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CHRONIC EFFECTS OF ENDRIN ON BLUNTNOSE MINNOWS AND GUPPIES

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CHRONIC EFFECTS OF ENDRIN ON BLUNTNOSE MINNOWS AND GUPPIES

Donald Irvin Mount

RESEARCH REPORT 58

UNITED STATES DEPARTMENT OF THE INTERIOR
Stewart L. Udall, *Secretary*
Frank P. Briggs, *Assistant Secretary for Fish and Wildlife*

FISH AND WILDLIFE SERVICE
Clarence F. Pautzke, *Commissioner*

BUREAU OF SPORT FISHERIES AND WILDLIFE
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ABSTRACT

In an investigation of chronic toxicity of endrin to bluntnose minnows and guppies, conducted for 291 days, neither species could withstand concentrations greater than 0.5 parts per billion of endrin in water for more than a few days. Less than 50 percent of the test animals could live in 0.5 p.p.b. for more than 30 days. Approximately 65 percent could live in 0.4 p.p.b. for periods exceeding 30 days, while there was little mortality due to endrin in 0.25 and 0.1 p.p.b. endrin in water.

Fish suffering from endrin poisoning exhibited symptoms (increased ventilation rate, convulsions, and loss of equilibrium) which indicate that it affects the central nervous system. Sublethal concentration caused increased activity and hypersensitivity to stimuli. No cell damage was found in fish which were able to survive in endrin concentrations, and essentially no physical effects other than increased activity were noted. Adult female guppies ceased to have young when placed in endrin concentrations, but the gonads of the bluntnose minnows were approaching sexual maturity when the test was terminated.

In continuous-flow acute-toxicity tests, it was found that the 96-hour TL_m values ranged from 0.27 p.p.b., for fish 30 mm. standard length, to 0.47 p.p.b., for fish 60 mm. standard length.

Endrin had no effect on oxygen consumption in the seven bluntnose minnows tested. Neither did acute exposure to endrin significantly affect the ability of the fish to swim against a current of water.

In carp exposed to concentrations of 2.5 to 10 p.p.b. endrin during periods varying from 2.5 to 28 days, the digestive tract, liver, heart-spleen-blood, and kidney contained the highest accumulations of endrin. The maximum concentrations of endrin found in the tissues were approximately 160 times greater than that in the water in which the fish were living. The concentration of endrin in the heart-spleen of a carp exposed 28 days to 2.5 p.p.b. was found to be 400 p.p.b. Muscle tissue was consistently low in endrin content. The gills were always low or negative in endrin content, whereas the digestive tract was consistently high, indicating that endrin probably enters the body through the intestine.

Endrin does not appear to be cumulative in its effects on guppies and on bluntnose minnows. Little or no tissue damage was found in fish which could live in water containing endrin, and fish seemed to recover completely from one exposure to endrin. The increased activity caused by very low concentrations of endrin could be very damaging to fishes in natural waters, disrupting spawning and subjecting the fish to predation and other decimating factors.

CHRONIC EFFECTS OF ENDRIN ON BLUNTNOSE MINNOWS AND GUPPIES

By Donald Irvin Mount

Ohio State University

In the past 15 years, the use of pesticides has increased rapidly, and the number of compounds used for pest control has grown at an astounding rate. Abstracting journals in recent years have had to add a subtitle for articles about pesticides, and each year this section becomes larger. Herbicides have increased in use in a similar manner, and at present are replacing cultivation in the production of some agricultural crops (corn, for instance). On the commercial market, one finds a whole array of compounds for combating roaches, ants, spiders, mites, and other pests. Cities now have spraying programs in which every alley is sprayed for fly and mosquito control. Highway departments spray roadsides with herbicides instead of mowing. The Federal and State governments, often in cooperation with local governments or agencies, spray large areas for various insect pests. The use of chemicals, especially DDT, for the control of forest pests is of ever-increasing importance.

Recently a huge aerial-spraying program has been conducted in the southeastern United States in an attempt to control the imported fire ant. Various exterminating agencies now use new chemicals to control termite damage to buildings, and often give a guaranteed protection period which may last several years. Marine paints on the market contain chemicals to control arthropod damage to wooden boats; floor waxes contain pesticides for ant and roach control. Some insecticides have been found very effective in rodent control, especially in orchards. Many conservation departments use chemicals to eradicate fish populations in large bodies of water, and it appears at present that effective control of the sea lamprey in the Great Lakes may be achieved by use of chemicals. In short, one will find pesticides used in almost any place he may go.

Such widespread use has caused many people to wonder what effect these chemicals may have on the intricate balance of animal populations. Unfortunately, many of the pesticides used are not very specific in their effects. These pesticides may alter food chains by removing a certain group of animals. A long residual life as well as high toxicity are sought-after features of pesticides, and for extended periods of time after spraying they are dangerous to animals other than those being controlled.

Aquatic animals in particular are highly susceptible to pesticides. As an example, Fitzwater (1958) reports that in 1953 an airplane carrying a spray mixture containing a little over 1 gallon of actual endrin crashed into a Louisiana bayou. This resulted in the death of all fishes and reptiles, including 42 alligators, for a distance of 35 miles from the point of the crash. Aquatic animals are often exposed

to insecticides as a result of runoff from treated areas, as well as from direct application to the water.

There is a dearth of information on the residual life of insecticides in soils and in organisms. Young and Nicholson (1951) reported what they thought to be the first recorded large-scale kill of aquatic animals caused by agricultural use of insecticides. There were 15 major fish kills in northern Alabama in one summer, and in Flint Creek in Alabama, in a 13-mile section varying from 6 to 20 feet in depth, there was an apparent total eradication of the fish population. These kills were thought to be due to frequent applications of insecticides, followed by several heavy rains in August which washed the insecticides from the soil into the streams. Barker (1958) found robins in Illinois dying from feeding on earthworms after heavy rains in the spring. Analysis of the worms showed high concentrations of DDT, which had been sprayed on the area almost 1 year earlier. Barker found the DDT content of the soil to increase in the autumn, presumably owing to the residue on the falling leaves. Barlow and Hadaway (1955) found DDT recoverable from soil 12 months after sorption was complete. Harrington and Bidlingmayer (1958) found extensive kills of aquatic animals resulting from aerial applications of 1 pound of dieldrin pellets per acre in a tidal marsh. They noted crabs eating moribund fishes and dying themselves the next day.

Hunt and Bischoff (1960) have presented a very complete picture of the accumulation of DDD (dichloro-diphenyl-dichloroethane) in animal tissues. In this case, Clear Lake in California was treated three times with DDD. The first treatment was applied in 1949 at a concentration of 1 part in 70 million, the second in 1951 at 1 part in 50 million, and the third in 1957 at the same concentration as the second. They found concentrations as high as 2,275 parts per million in the fat of apparently healthy largemouth bass, and concentrations as high as 42 parts per million in the fat of yearling largemouth bass hatched 7 to 9 months after the last application. Carnivorous fish had higher levels of DDD in their tissue than did plankton feeders.

All these investigations and many more not cited point up the need for cautious use of insecticides, and for extensive studies to determine what the total effect of their use may be over a long period.

Certainly in coming years, as the world population increases, the value of our inland waters for recreation as well as food supply will be increasingly important, and the aquatic habitat must be protected. At the present increased rate of use of pesticides, it is not impossible that even the oceans along the continental coasts may receive enough to reach a toxic level. The unfortunate fact is that in general there is little concern about the danger until desirable organisms begin to die, and often it is then too late to remedy the situation.

Acute testing of new chemicals on various animals is rapidly becoming standard procedure before the chemicals are released to the public. Toxicity is usually measured by the number of deaths, but sublethal effects also can be very damaging. The need for chronic-toxicity studies is becoming more pressing and is gaining some support. The importance of finding symptoms of poisoning at a time when complete recovery is still possible is slowly being recognized. In an effort to focus attention on this area, as well as to determine some of the effects of sublethal doses, an investigation of the chronic toxicity of endrin to fishes was initiated. Endrin was chosen because of its extreme toxicity to fishes and its increasing use as a pesticide. Henderson, Pickering, and Tarzwell (1959) state, "Endrin was by far

the most toxic of the insecticides to all species of fish [tested]. In fact, it is the most toxic chemical that has been tested in this laboratory." They found endrin to be lethal in 96 hours to 50 percent of the bluegills tested at a concentration of 0.6 p.p.b. (parts per billion) in the water.

Endrin is 1,2,3,4,10,10-hexachloro-*exo*-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*endo*-5-8-dimethanonaphthalene. It is a yellowish-white crystal with a sweet odor when it is in a dry form. Endrin is slightly soluble in water and moderately soluble in alcohol.

The use of endrin has increased each year since it was first introduced in the early 1950's. Officials of one agricultural chemical company informed me that they sold several thousand pounds of active endrin in a 3-State area during 1958. Endrin has been used effectively to control many pests. A few are listed below to illustrate its broad spectrum of toxicity.

Other pests such as army cutworms, plant-lice, tent caterpillars, and white-pine weevils have been effectively controlled.

Although such application rates may seem low, Tarzwell and Henderson (1956) working with a closely related compound, dieldrin, found runoff from the first and third rainfalls from an area treated with 4.66 pounds of dieldrin per acre to be lethal to 50 percent of the bioassay fish in 96 hours. Runoff from the fourth rain still contained some dieldrin, as indicated by the reactions of fish placed in a sample of it. Rosen and Middleton (1959), by use of a carbon filter, found 0.1 and 0.013 p.p.m. in the runoffs from the first and third rains in the treated area mentioned above. These two experiments, together with many published accounts of fish mortality, indicate that these insecticides do reach the streams in sufficient quantity to be toxic. Mr. Henderson has informed me that "quite constant amounts" of insecticides are found in some streams. Undoubtedly the concentrations increase in periods of widespread spraying (April and May), but it is significant that there is a measurable level of insecticides in the streams at all times. Presumably these levels are maintained by residues of insecticides in the soil which have been shown to last for at least 1 year (Barlow and Hadaway, 1955). Seasonal spraying renews this supply in the soil. This fact makes the need for studies of chronic effects even more pressing.

A literature search revealed no studies on the chronic effects of endrin on fishes, although some experiments have been conducted on birds and mammals. Treon, Cleveland, and Cappel (1955) found that all rats fatally poisoned by endrin had "diffuse degenerative changes in the liver, kidney, and brain." In a rabbit given 50 oral doses (1 mg./kl.), endrin caused "diffuse degeneration and fatty vacuolation of hepatic and renal cells and degeneration of the heart." Survival times of male rats fed 100 and 50 p.p.m. endrin in their food, and of females fed 100, 50, and 25 p.p.m., for periods up to 106 weeks were significantly shorter than for those animals fed 1 and 5 p.p.m. Concentrations of 50 and 100 p.p.m. in food caused the ratio between liver weight and body weight to be larger

USE OF ENDRIN TO CONTROL CROP PESTS

Pest	Crop	Dosage
Spiny bollworn	Cotton	500 grams per hectare.
Lesser peach borer	Peaches	1 qt. emulsified endrin per 100 gal. (0.5 gal. per tree.)
Potato tuber moth	Potatoes	0.25-percent spray.
Red-banded thrips		1 pt. per 100 gal.
Potato leafhopper	Potatoes	2 cc. per gallon of 19.5-percent endrin.
Citrus psylla	Citrus fruit	0.01-percent endrin.
Pine mice	Orchards	2.5 lbs. per 52 trees.

than normal. Male rats fed 5 and 25 p.p.m. for 2 years also showed an increased liver weight to body weight ratio.

They also stated that, in general, the only effect in female dogs fed 4, 3, and 1 p.p.m. for 6 to 19 months was some slight renal tubular vacuolation. Some dogs fed 3 p.p.m. for 19 months had enlarged kidneys and hearts. Those dogs which survived on 8 p.p.m. had normal viscera, except for slight diffuse degeneration of the distal convoluted tubules in the kidneys.

DeWitt (1956) reported that 10 p.p.m. endrin fed to adult pheasants during the reproductive period reduced hatchability of eggs and survival of the young. One p.p.m. fed to quail before the reproductive season, but discontinued during reproduction, reduced the fertility and number of eggs, as well as the survival of the young.

Sherman and Rosenberg (1953) found 3.5 mg./kg. of endrin in 7-day chickens to be lethal between 6 hours and 5 days. It caused degeneration of the liver and kidney, and following long exposures was stored in peritoneal fat and the liver.

Kunze and Laug (1953) detected storage of endrin in the brain and liver of rats. Females contained more fat-stored endrin than males. These investigators recovered considerable amounts of endrin from the kidneys, but this was of a "different chemical nature."

Ely, Moore, Carter, and App (1957) reported that 20 mg. or more per day fed to cattle caused measurable amounts of endrin to occur in the milk. One and one-half milligrams per kilogram per day caused the cattle to exhibit toxic symptoms.

Data on the effect of endrin on fish are very scanty. Henderson, Pickering, and Tarzwell (1959) state that in soft water (alkalinity 18 p.p.m., hardness 20 p.p.m.) at 25°C. the 96-hour TL_m (median tolerance limit) was 0.6 p.p.b. for bluegills, *Lepomis macrochirus*, 1 p.p.b. for fathead minnows, *Pimephales promelas*, and 1.9 p.p.b. for goldfish, *Carassius auratus*. These fishes were 1.5 to 2.5 inches long. Guppies, *Lebistes reticulatus*, 0.75 to 1 inch long, had a 96-hour TL_m value of 1.5 p.p.b. under the above-mentioned conditions. These were static tests with five guppies in 2 liters of test solution and five fishes in 10 liters of solution for the other three species. In constant-flow tests of 20 days' duration, they found endrin to be toxic at levels of half the 96-hour TL_m value. Their conclusion was that endrin had a cumulative or chronic effect on fishes. They concluded also that natural variations in pH, alkalinity, and hardness have no major effect on the toxicity of the chlorinated hydrocarbon insecticides to fishes, although endrin, among others, was slightly more toxic in soft water.

Iyatomi et al. (1958) determined the 48-hour TL_m for 5- to 7-centimeter carp to range from 140 p.p.b. at 7°–8° C. to 5 p.p.b. at 27°–28° C. They found that the 24-hour TL_m for day-old carp was 8,500 p.p.b., which decreased to 46 p.p.b. at 6 days of age. It is quite probable that these TL_m values are high, since five fishes (5 to 7 centimeters long) were placed in 2 liters of water. Such large fish in 2 liters of water would absorb endrin and thereby reduce the concentration.

Both of the last two papers mentioned considered the effect of the form in which the endrin was administered (powdered, granular, or emulsion) on the lethal dose. Form was not considered in the current investigation. Form probably affects the amount dissolved in the water and not the actual toxicity.

Objectives

The objectives of this investigation were—

1. To determine the maximum concentration in which guppies and bluntnose

minnows could survive for extended periods.

2. To determine reproductive and physiological effects of sublethal concentrations of endrin on fishes.

3. To determine the place of entry into, and the movement through, the fish's body.

4. To determine any differences in the acute toxicity of endrin to various size groups.

5. To determine whether there is a potential danger to human beings or to other animals which eat fishes that have been exposed to sublethal doses of endrin.

The methods and equipment employed in this investigation are described separately with each phase of the investigation. Raw data are given in the appendix. As used here, the terms "chronic test" and "acute test" refer to tests of long and short duration, respectively.

This report was prepared in partial fulfillment of the requirements for the degree of doctor of philosophy in the Graduate School of Ohio State University. Dr. Wilson Britt was my major advisor and Dr. Eugene Dustman was my coadvisor during my doctoral study. In addition, I am indebted to Drs. William Spoor, David F. Miller, George Ware, Frank Fisk, and William McIntosh for advice and assistance.

CHRONIC-TOXICITY TESTS ON BLUNTNOSE MINNOWS

Objective

The objective of this experiment was to determine the effects of sublethal levels of endrin on bluntnose minnows exposed for extended periods of time.

Methods and equipment

The chronic-toxicity testing was based on a continuous flow system. The apparatus consisted of a reservoir capable of holding a 4-day supply of the test solution, a constant-head chamber, and a test chamber, all shown in figures 1 and 2.

The reservoirs were made of ¾-inch marine plywood coated with four coats of plastic resin in which glass cloth was imbedded for strength (Mount, 1959). Ten such reservoirs, 16 by 16 by 24 inches, were arranged in two batteries of five each. They were supported 4 feet above the floor by six 4-by-4 legs and eight 2-by-6 planks. Glass tubing was bent as shown in figure 2 to serve as water-level gauges.

Water was siphoned from the reservoirs into a constant-level chamber. Figure 3 shows the details of the float valve used to control the water flow into the constant-level chamber; the level did not vary by more than ¼ inch during operation. Automotive valve-grinding compound was used to seat the ground-glass surfaces. No sealing compound was used on the valve seats; only a slow seepage occurred when the valve was closed. Large cork stoppers coated with plastic resin served as floats. Ten of these valves functioned for periods of 6 to 11 months with approximately 10 failures due to particles of dirt.

Water was siphoned from the constant-level chamber into the test chamber, and the flow was controlled by small glass jets. These jets were made by heating and drawing glass tubing to a very small diameter. The jet was fastened to the water line, and small pieces were broken off until the desired flow rate was obtained. Glass stopcocks and screw clamps were tried, but both failed to maintain a constant water flow and became clogged quickly with dust particles. Flow rates were measured by a 10-milliliter graduated cylinder. All water lines were made of glass tubing with tygon tubing at the joints.

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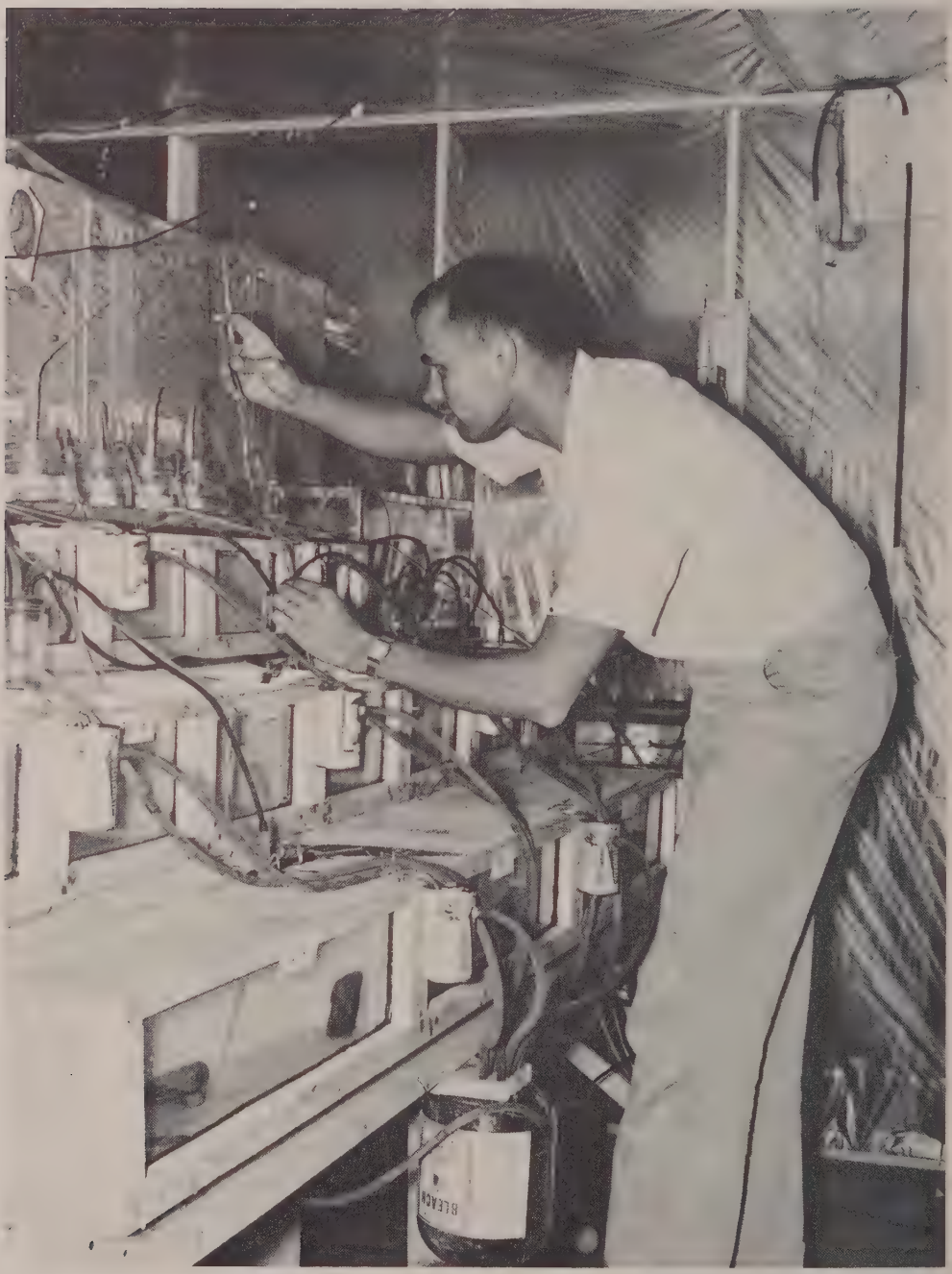

FIGURE 1.—Continuous-flow system used for the chronic-toxicity testing.

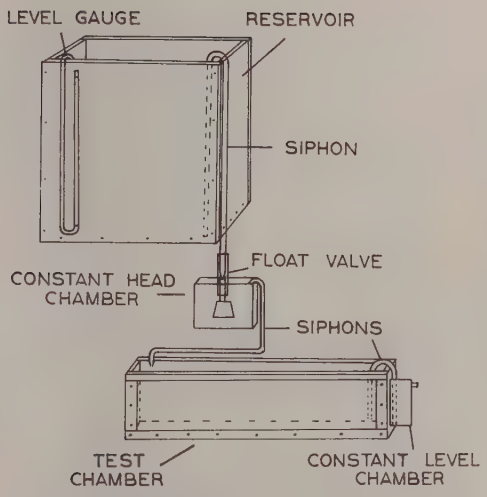

FIGURE 2.—Diagram of continuous-flow system used for chronic-toxicity testing.

The bluntnose-minnow test chambers, 10 by 24 by 6 inches, were constructed in the same way as the reservoirs, except that the front was glass, and white pigment was added to the plastic resin. A narrow retaining strip was placed around the front to hold the glass in place. Double-strength window glass was cut so that it was as large as the entire front, and was sealed in place with aquarium cement. A strip of aquarium cement ⅛ inch wide or less, around the glass, was exposed to the test solution. Each test chamber, when filled to normal level, had a capacity of approximately 18 liters. A U-shaped glass tube siphoned the water from the test chamber into a constant-level chamber, and from there it was emptied into the drain. The end of the tube in the test chamber was inserted into a cylinder made of plastic screen in order to prevent fish from entering the siphon tube. The test chambers were joined in batteries of three to seven, and arranged in three banks or levels, all lower than the reservoirs. Compressed air was bubbled into the water in each chamber through a glass jet.

Since the endrin was added directly to the reservoir, the level gauge of each reservoir was calibrated so that exactly 80 liters of water could be measured into it. In this way the desired concentration could be made directly in the reservoir. There were 2 inches of water under the zero level mark in the level gauge, so that when the reservoir was lowered 80 liters the columns of water in the level gauge and siphon tube were not broken. A change in volume of 0.1 percent could be detected on the level gauge. Each reservoir held a 4.5-day supply of solution which flowed out at the rate of 720 ml. per hour. This rate amounted to the approximate capacity of the test chamber in 24 hours.

In 1.5 hours, all 10 reservoirs could be drained and refilled with fresh solutions. During the filling, the siphon tubes were clamped to prevent an improper concentration from reaching the test chambers.

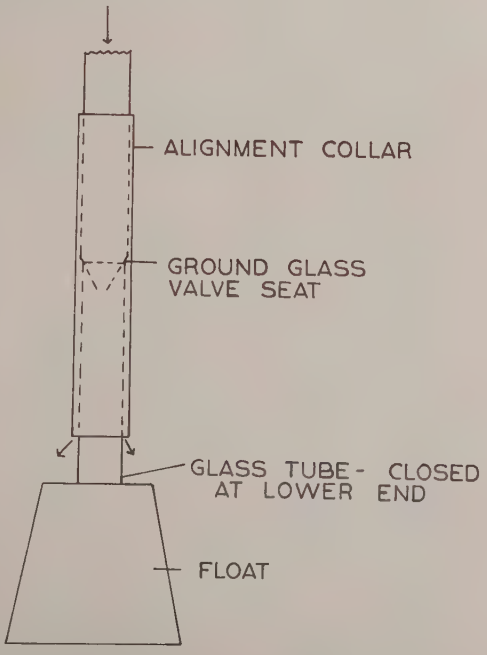

FIGURE 3.—Float-valve assembly for regulating water-flow.

This entire unit was set up in a basement room built especially for the experiment. The walls were made of two sheets of polyethylene-plastic film with 4 inches of dead-air space between them. Sunlight from a basement window entered through the plastic wall; thermostatically controlled electric heaters held the temperature constant. It was found that small variations in air temperature did not alter water temperature measurably. There was temperature stratification of the air; but this proved to be advantageous because the water in the reservoirs was 1° to 2° C. warmer than that in the test chambers. Influent was introduced at the water surface in the test chamber, and since it was less dense it spread out over the surface and settled to the bottom as it cooled. The outlet siphon drew the water from the bottom. This movement, plus the turbulence from aeration, gave good water circulation through the test chamber as determined by methylene-blue dye. Four test chambers and four reservoirs were used for the bluntnose-minnow tests. Three test chambers contained endrin and were designated TB–6, TB–7, and TB–8; one designated CB–10, without endrin, served as a control.

Physical and chemical conditions

The chronic-toxicity tests were conducted at a constant temperature of 22.2° C., plus or minus 1° C. The summer of 1959 was unusually warm, and on several days the capacity of the cooling unit was exceeded. For 3 days in August, the temperature reached 25° C. in the late afternoon, and for a total of 18 days in the months of June, July, and August the temperature exceeded 23.2° C. but did not reach 25° C. I feel that this variation did not affect the results significantly, since this was a chronic test, and because any variation due to temperature fluctuation would affect the control group as well as the test fishes. During all months except June, July, and August the temperature rarely varied by more than plus or minus 0.5° C. Continuous temperature records were kept by a recording thermograph. Examination of these records showed that no large temperature fluctuations occurred at any time.

Water chemistry in the test chambers was determined periodically, and the pH and alkalinity of the tap water were measured each time before the reservoirs were filled. Chemical analyses of the tap water upon leaving the Morse Road water-treatment plant are shown in table 1. This information was made available through the courtesy of Mr. Richard Melick, Division of Water, Columbus, Ohio. An analysis by the State health department on November 19, 1959, showed, in addition, fluorides 0.2 p.p.m., silica 4 p.p.m., nitrate nitrogen 0.5 p.p.m., and sodium 7 p.p.m.

TABLE 1.—*Chemical analyses of Columbus tap water*

[All values except pH are expressed in p.p.m. These values were determined by the Columbus Division of Water on the water as it left the Morse Road treatment plant]

	Oct. 21, 1959	Nov. 25, 1959	Dec. 28, 1959	Feb. 2, 1960
Total alkalinity	22	22	20	21
Phenolphthalein alkalinity	4	3	12	10
Noncarbonate hardness	52	54	62	71
Total hardness	74	76	82	92
Calcium	18	21	24	26
Magnesium	7.5	6	5	7
Iron	0	0	0	0
Chlorides	7.5	8	7.5	9.5
Sulphates	52	58	62	66
Free CO_2	0	0	0	0
pH value	10.1	8.5	10.2	10.0
Turbidity	.2	.25	0	0
Total solids	153	139	148	166
Manganese	.3	0	0	0

According to the analysis made at my laboratory before the reservoirs were filled, the chemical characteristics of the tap water used in the tests, based on 73 samples over a period from April 11, 1959, to January 27, 1960, were as follows:

	Range	*Mean*
pH	6.5– 8.5 p.p.m.	7.8 p.p.m.
Total alkalinity	15.0–42.0 p.p.m.	23.9 p.p.m.

Only two alkalinity determinations exceeded 30 p.p.m., and only two pH readings were below 7.0. Phenolphthalein alkalinity was always present in tap water, but disappeared during aeration as the reservoirs were filled. Free chlorine appeared in the water for the first time on November 29, 1959, and filtering through charcoal was necessary from then until the end of the test. The filter consisted of a 4-foot length of glass tubing, 100 mm. inside diameter, packed with granular bone charcoal. Chlorine determinations were made according to the starch-iodine test described in Standard Methods for the Examination of Water and Sewage (1949).

Table 2 summarizes the chemical characteristics of the solutions in the test chambers. No phenolphthalein alkalinity was found in the test chambers. The unmodified Winkler method was used for dissolved-oxygen determinations, and dissolved carbon dioxide and alkalinity were determined by standard methods described by Welch (1948). Indicator solutions with a color chart were used to determine pH. A comparison of this method with a pH meter indicated that the method was sensitive to 0.25 pH units.

The chronic testing was begun on April 11, 1959, and was conducted for 291 days, ending on January 27, 1960. Five bluntnose minnows, *Pimephales notatus*, were placed in each of the four test chambers. They were assigned to chambers at random by the use of a table of random digits. Rocks of similar composition were placed in each chamber to serve as cover. Hardware cloth of ¼-inch mesh was placed over the top to keep the fish from jumping out. Ground commercial dog-chow checkers were free-fed twice daily. This diet was supplemented from time to time with chopped earthworms taken from an area known to be free from insecticides. The fish were overfed intentionally, so that all fish were able to eat as much as they would. The excess food and excrement were siphoned daily.

This species fights very little under laboratory conditions, and its behavior, plus its tolerance to laboratory conditions, makes it a very desirable test animal. All fish used in the chronic test were collected in a small pool in Rocky Fork Creek, on the Franklin-Delaware County line, on March 16, 1959. The fish were held in the laboratory at 22° C. for 26 days before the test for chronic effects was begun. Those not used immediately in the tests were held at 22° C. for later use.

TABLE 2.—*Chemical analyses of water in the test chambers based on determinations made approximately every 9 days during the 291-day test period*

[All values except pH are expressed in p.p.m.]

Chemical character	Range	Mean
Total alkalinity	18–33 (One reading over 28)	24.0
Dissolved oxygen	5.3–7.2	6.2
Dissolved CO_2	1–3	2.3
pH	7.0–7.8	7.4

Ninety-six percent pure reference standard endrin was used in the tests. A stock solution of 1 mg. endrin per milliliter of 95-percent ethyl alcohol was prepared, and the desired amounts were measured with a 1–milliliter-graduated pipette. Initially, 200 ml. of the stock solution were prepared. After this had been used, one liter of solution was made and this was sufficient for the rest of the chronic test and for all of the acute testing.

When filling the reservoirs, I dropped the appropriate amount of the stock solution into a beaker containing approximately 100 ml. of water. The beaker was then emptied into the reservoir and washed for several minutes under the water stream entering the reservoir. It was necessary to keep the alcohol content as low as possible, because it permitted a considerable growth of bacteria, even in low concentrations. A volume of pure

95-percent ethyl alcohol, equal to the largest volume of alcohol put into the test chamber of highest endrin concentration, was put in the control chamber. This originally amounted to 0.72 ml. per 80 liters of water, but later when the maximum test concentration was dropped to 0.5 p.p.b. endrin, it was decreased to 0.4 ml. per 80 liters of water. Even this amount permitted an appreciably greater bacterial growth than did the 0.08 ml. put into TB–8. The stock solution was measured near the floor to avoid splashing endrin into the test chambers. The bottle containing the stock solution, and the pipette, were frequently wiped with a cloth saturated with pure 95-percent alcohol to remove any traces of endrin residue.

During the testing period the need for determining the concentration of endrin in the test chambers became apparent. The best chemical test for endrin is sensitive only to 5 p.p.b., but by bioassay it was possible to determine concentrations as low as 0.4 p.p.b. In November 1959, during acute-toxicity testing, it was found that 100 percent of the fish 20 to 40 mm. standard length would die in concentrations as low as .4 p.p.b. in less than 4 days. Fish of similar sensitivity were used to determine the concentrations in the test chambers by bioassay techniques. In acute tests in which the concentration was better known, fish died in 1 to 2 days at 0.6 p.p.b. and in 2 to 3 days at 0.4 p.p.b.

A 5-gallon glass jug with the top cut off was placed so that the effluent from TB–7 flowed through it. Later, this was also done with TB–6 and with the influent to each of these chambers. The results are summarized in table 3. By comparison with known concentrations, these data show that the concentration in TB–7 was approximately 0.5 p.p.b., while the concentration in TB–6 seems to have been somewhat lower than 0.4 p.p.b. Since these concentrations are very near the threshold of tolerance, the fact that no fish died in the TB–6 effluent may not be significant. After the chronic test was ended, five fish ranging from 29 to 38 mm. standard length were placed directly into TB–7, and all died within 3 days. Five others were placed in TB–6. One disappeared, but the other four died within 3 days, indicating that the concentrations were as expected. (Despite precautions, fish were occasionally lost by jumping out onto the floor.) Although the actual concentrations may have been slightly lower than the calculated concentrations in some chambers, this does not introduce serious errors because the concentrations in TB–6 and TB–7 were the maximum that the fish could withstand.

The small loss of endrin may have been a result of two factors: absorption by the fish and loss because of aeration. The manufacturers both of the plastic resin used to coat the reservoirs and chambers, and of the endrin, gave assurance that there would be no reaction between the endrin and the plastic. The concentration was changed in several chambers and, so long as they were washed thoroughly with soap and water, there was no residual insecticide found in the water.

TABLE 3.—*Bioassay of test concentrations of the chronic-toxicity tests*

[Young bluntnose minnows were used as bioassay animals. Survival rates in known concentrations are taken from acute-testing results. See appendix table 1]

Source of water	Number of fish	Size range, mm. S.L.	Average survival in days
Effluent from TB–7	5	25–30	2.2
Do	4	54–59	4.0
Influent to TB–7	5	22–33	2.4
Effluent from TB–6	5	25–30	(1)
Influent to TB–6	3	29–35	5.3
Known concentration, .6 p.p.b.	5	29–34	1.0
Do	5	26–31	2.0
Known concentration, .4 p.p.b.	5	25–34	2.4
Do	5	26–32	3.0

[1] No deaths.

In order to determine the effect of aeration on the loss of endrin from water, 9

liters of a 5-p.p.b. solution were made and divided equally into two battery jars. One was aerated vigorously with five air jets for 72 hours; the other solution was not aerated. At the end of 72 hours, five fish were put into each jar. All fish died in 12 hours in the unaerated solution, but none died within 48 hours in the aerated one. This evidence indicates that aeration does remove at least some endrin. Aeration was very slow in the test chambers and previously cited evidence indicates that there was a minimal loss.

Results

Initial test concentrations were selected on the basis of 48-hour screening tests. Concentrations of 0.9, 0.5 and 0.1 p.p.b. were used for chronic testing. The survival times are listed in table 4. As fish died in the various chambers, other fish were fin-clipped for identification and were placed in the chambers to maintain five animals per chamber.

A summary of the growth data on fish living for extended periods is given in table 5. Means are expressed as individual growth per fish per day. The mean for each group was calculated as follows:

$\frac{G}{T}$=mean daily growth.

T=Total number of days that n fish lived.

G=Total growth of n fish.

n=Number of fish in the group.

Discussion

Fish poisoned by endrin exhibited symptoms clearly indicative of central-nervous-system disorders. The first indication of poisoning was an increase in ventilation rate accompanied by rapid, jerky movements of the body and fins. Almost simultaneously, an increased sensitivity to external stimuli would be noticeable. Next, the fish would move to the surface, and shortly thereafter begin turning

TABLE 4.—*Summary of the chronic-toxicity tests on bluntnose minnows*

Concentration of endrin	Chamber	Sex	Initial size (mm. S.L.)	Number of days lived	Growth (mm.)
0.9 p.p.b.	TB-6	F	46	9	
		F	36	11	
		M	42	6	
			42	3	
			36	5	
			36	4	
0.75 p.p.b.	TB-6	F	41	9	
		M	36	9	
		M	37	10	
		M	38	29	
			38	9	
0.5 p.p.b.	TB-7	F	50	81	
		F	36	38	
		F	49	[1] 290	8
		F	35	[1] 291	23
		F	56	7	
		F	57	11	
		F	60	4	
		F	45	7	
		F	48	[1] 106	3
		M	43	17	
		M	51	[1] 106	5
		M	45	[1] 105	5
			33	11	
			38	6	
0.4 p.p.b.	TB-6	F	59	[1] 194	0
		F	39	196	13
		F	45	10	
		F	39	[1] 107	3
		M	53	[1] 231	19
		M	47	[1] 196	8
			55	11	
			37	8	
			36	10	
			36	12	
0.1 p.p.b.	TB-8	M	50	247	21
		M	49	[1] 291	23
		M	37	[1] 291	32
		M	35	[1] 291	33
		M	31	[1] 291	35
0 p.p.b.	CB-10	F	45	274	14
		F	35	269	23
		F	36	[1] 291	19
		M	69	[1] 51	5
		M	47	[1] 291	20
			39	[2] 62	

[1] Killed at the end of the test.
[2] Jumped out of test chamber.

TABLE 5.—*Mean daily growth of bluntnose minnows*

[In millimeters. Number in parentheses is number of fish on which the mean is based]

Concentration of endrin	Chamber	Mean daily growth of—		
		Males	Females	Both sexes
0.5 p.p.b.	TB-7	0.047 (2)	0.050 (3)	0.049 (5)
0.4 p.p.b.	TB-6	.063 (2)	.032 (3)	.047 (5)
0.1 p.p.b.	TB-8	.102 (5)		.102 (5)
0 p.p.b.	CB-10	.073 (2)	.067 (3)	.069 (5)

around and around rather slowly, frequently swimming backwards. Convulsions would follow, and between them the fish would vibrate. The fins and body were not still for a second, and the fish jumped out of the water frequently. Convulsions would become more severe and of longer duration; finally, loss of equilibrium would occur. The fish often lived several hours after loss of equilibrium, and would make periodic darts around the chamber, often "barrel rolling" in the process. Breathing would then stop, but usually the heart would continue to beat for 10 to 15 minutes. The body would be limp at this time, but as rigor mortis set in, a characteristic bend in the spine would occur at about the region of the anus, so that the body formed an obtuse angle. During the period of poisoning, there was a progressive darkening of body coloration until the fish died.

In weaker concentrations, the above symptoms were prolonged over several weeks. Some fish were in the stage of hyperactivity for many days. The two females that lived 290 and 291 days in 0.5 p.p.b. (table 4) were in such a state for 2 months. They became emaciated; their abdomens caved in so that they appeared humpbacked. (This may have been an illusion due to the hollow abdomens.) When food was dropped into the chamber, they attempted to feed, but usually they were unable to grasp the food, often missing it completely, as if their coordination were impaired. For a period of several weeks they ate little if anything. On June 10, 1959, two fish were put into the chamber with them. The latter two died within 11 days, but the original two females began to feed, and by July 1, had begun to gain weight and appeared normal. The behavior of these females during the first 2 months was typical of many other fish which had a prolonged period of poisoning before death.

Because of the limited facilities, only a very small number of fish could be used in the test; therefore, any conclusions drawn from the data must be made with caution. Small numbers were employed to avoid crowding with the hope that under these conditions the fish would spawn.

The high growth rates of those fish in 0.1 p.p.b. stand out as the most significant difference in table 5. This growth was greater than that of the controls, and began very early in the test period. Fish were not measured during the test period because of the danger of injury. Unfortunately, all the fish in 0.1 p.p.b. were males, and the higher growth rate may be due to this fact. It is true, however, that the females grew more than the males in one out of three chambers in which both sexes were present. This is an indication that the larger length-increase in 0.1 p.p.b. endrin was not attributable to the fact that all were males. One might postulate many reasons for this growth rate, but the significant point is that these fish were able to grow in 0.1 p.p.b. endrin and, as will be pointed out later, showed some indication of reproductive activity.

The fish in 0.1 p.p.b. endrin appeared to be healthy, and behaved in a normal manner. They fed readily as soon as food was introduced. When they were examined after being killed, there were many fat deposits, especially around the abdominal cavity. These fish were much fatter than any of the other fish tested, including the controls. Whether there was any relation between this growth and the endrin is not known. This effect should be tested further with a larger number of fish.

If we assume similar growth rates for both sexes, the combined mean growth increments can be compared. In 0.5 and 0.4 p.p.b., the growth rates were lower than the control group by factors of approximately 0.71 and 0.68 respectively. These two concentrations were the ones in

which a number of fish died before five specimens were found that would live in them, whereas the first five put into 0.1 p.p.b. lived without visible signs of poisoning. Reference to table 4 will show that in 0.5 p.p.b. 9 out of 14 or 64.3 percent died, and that 5 out of 10 or 50 percent died in 0.4 p.p.b. These percentages approximate the 96–hour $TL_{\overline{m}}$ values determined in the acute-toxicity testing. DeWitt et al. (1960) stated that in pheasants the concentrations of endrin producing acute and chronic effects were almost identical. A comparison of table 4 and table 9 indicates that the concentrations producing chronic and acute effects were very close together for the bluntnose minnow. In fact, those producing chronic effects are a little higher. This is thought to be attributable to the time of year, and to variations in individual fishes.

There were five deaths which occurred near the end of the chronic testing. A male in 0.1 p.p.b. appeared to be in breeding condition before losing equilibrium after 247 days in the test chamber. An autopsy showed excessive stored fat in all body parts, and sections of the liver revealed almost complete fatty vacuolation of the liver. When sections of the spleen, kidney, heart, and brain were compared with sections from normal tissue, no difference was noted.

Four females from the other test chambers died in the last 20 days of the test. They exhibited bulging eyes and rapid ventilation rates before death. The sectioned viscera appeared normal, the ovaries were well developed, and there were considerable fat deposits.

Sections of the liver and gonads of all fish which were living at the end of the test were prepared using the paraffin method and staining with hemotoxylin and eosin. Most of the fish, except those in 0.4 p.p.b., had considerable lipid deposits in the cells of the liver. In some cases, 90 percent of the surface area of a section was blank space, which I assume represents lipid material removed in the alcohol dehydration process. Sections of liver from fish recently taken from the stream did not show such fat deposits. In view of the low liver-fat content found in fish in 0.4 p.p.b. (which were in the test chambers for shorter periods of time) and in fish taken directly from the stream, it seems probable that this fatty vacuolation was caused by a high lipid content in the diet.

The effect of diet and reproductive condition on stored fat is discussed by W. S. Hoar in Brown's Physiology of the Fishes (1957). According to this author, changes in liver size due to a change in fat content, and correlated with the seasons, has been observed in several species. Apparently, a high fat content is normal for some fishes, but whether or not the condition found in these fish was normal is not known.

One male in 0.4 p.p.b., one in 0.1 p.p.b., and one in the control group had spermatids in the testes and moderately well developed tubercles on the head. The other males had both primary and secondary spermatocytes in the testes. Fighting was common among the males in 0.1 p.p.b., indicating sexual activity.

All the females had ovaries in a state of maturity comparable to fishes taken in the stream. Several females in TB–6, TB–7, and CB–10 showed distention of the abdomen resulting from ovarian development. It appears, therefore, that endrin in concentrations of 0.5 p.p.b. or less will not affect gonadal development in individuals which can tolerate such concentrations. More data are needed on this aspect of the subject before conclusions can be reached. It must be emphasized that only a fraction of the fish were able to live in

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0.5 and 0.4 p.p.b. One fish in each of these concentrations was slightly emaciated, but no other physical effects were noted.

Little difference in activity was noted between fish in 0.1 p.p.b. and the control group, but fish in 0.5 and 0.4 p.p.b. rarely stayed under the rocks. On the other hand, the fish in 0.1 p.p.b. and in the control chamber normally stayed under the rocks during daylight hours. After sundown, all fish rested quietly on the bottom with little movement. Even fish having convulsions became quiescent after the room was darkened.

The increase in activity mentioned above could have a very detrimental effect on the fish in their natural environment. If sublethal concentrations of endrin were present in a natural body of water, they could quite possibly cause the fish to leave their normal habitat niche and be subjected to predation and the effects of current. The bluntnose spawns under objects, and such an effect on the activity could upset the spawning behavior and result in infertile eggs in this species. It is also important to bear in mind that even though the gonads were maturing, spawning did not occur, so that it cannot be concluded that endrin had no effect on spawning. These are matters that must be investigated before any conclusions can be drawn about the effects of sublethal concentrations on reproduction.

CHRONIC-TOXICITY TEST ON GUPPIES

Methods and equipment

This experiment was conducted simultaneously with the chronic-toxicity test on bluntnose minnows, from April 11, 1959, to October 24, 1959, under the same conditions. The water chemistry was the same as described earlier. The only differences were in the number and size of test chambers, and the endrin concentrations used. Guppy test chambers were 10 by 12 by 6 inches and held approximately 9 liters at test level; the flow rate was 6 ml. per minute. Paired chambers (designated by the letters "A" and "B" after the number) were fed by one reservoir and emptied through a common constant-level chamber. Test chambers were half as large as those used for bluntnose chambers; two test chambers could be supplied for the same period of time by one reservoir of the same size as those used for the bluntnose minnow tests. Six pairs of chambers (five pairs of test chambers and one pair for controls) were used in these tests.

The chronic-toxicity test was begun by assigning five 1- to 7-day-old guppies at random to the test chambers. Initially, endrin concentrations of 0.9, 0.75, 0.5, 0.25, and 0.1 p.p.b. were used. It was soon apparent that 0.9 and 0.75 p.p.b. were too high, so a 0.4 p.p.b. concentration was included. The guppies used were produced from breeders obtained from a local tropical-fish store.

Results

Before proceeding with the discussion of the data, it must be stated that the guppy test data should be interpreted cautiously because the animals became diseased, which probably affected some of the results. I first became aware of this disease early in September when the females in the control group began to appear dark in color, behave abnormally, and lose weight. They swam slowly about and stayed near the bottom, frequently with the head lower than the rest of the body. Immediately before they died, their abdomens became distended, their scales stood perpendicular to the body, and the fish came to the water surface. The same

symptoms appeared in fish in other chambers and were followed by death. Examination of the dead fish revealed that the kidneys were enlarged and that there was an edema of the body cavity. Axelrod and Schultz (1955, p. 142) describe a disease called dropsy, which has symptoms similar to those observed in these test fish. The cause of this disease is thought to be a primary virus infection accompanied by a secondary bacterial infection. Upon checking back in my records, I found that some of the recorded descriptions of dying fish indicated that deaths may have been due to the disease as far back as the first of August. The disease seemed to affect sexually maturing females first and then the males. Because of this disease, the chronic study on the guppies had to be discontinued on October 24, 1959.

The question arises how the results of the guppy tests should be treated. Table 6 lists the fish which died in 0.9 and 0.75 p.p.b. Since the fish in these tests did not live for long, and since these tests were completed at least a full month before the disease was first observed, the results are probably reasonably accurate. At the time these tests were conducted, all fish in all chambers appeared healthy and normal, and those in the lower concentrations of endrin and in the control chambers were growing normally.

Table 7 presents the data for the lower concentrations of endrin tested. Here the disease no doubt was an important factor. For instance, in 0.25 p.p.b. there is a rather uniform progression of deaths with time; this is probably due to the disease, since the more usual pattern of deaths due to endrin, in both species, was numerous deaths within the first few weeks and then long periods before other deaths occurred. In view of the disease factor, no data have been given on growth rates, other than to indicate the fishes which attained sexual maturity.

Perhaps the most significant point to be inferred from the data is that if diseased fish were able to grow and reach sexual maturity in various concentrations from 0.5 to 0.1 p.p.b., then normal fish ought to survive and grow in at least those concentrations, if not in slightly higher concentrations.

Discussion of data

Inspection of the data given in table 6 for the two highest concentrations indicates that the adults were significantly more tolerant than the young. The reasons for this increased tolerance in the larger fish are not known.

A comparison of mean survival times in all concentrations shows an inverse relation between concentration and survival. If the mean survival times (tables 6 and 7) are plotted against the logarithm of the concentration, the points fall along a straight line, which is often the case in toxicological studies.

TABLE 6.—*Survival of guppies in 0.9 and 0.75 p.p.b. endrin*

Initial age	Survival time of each fish in days	Mean survival in days
0.9 p.p.b. concentration of endrin:		
1–7 days	3–3–3–3–2	2.8
1–7 days	3–4–4–5–6	4.4
1–7 days	4–4–4–4	4.0
1–7 days	4–4–4–4–4	4.0
Adult males	7–9	8.0
Adult females	8–11–13	10.6
Mean for 1–7 day fish		3.8
0.75 p.p.b. concentration of endrin:		
1–7 days	3–3–4–4	3.5
1–7 days	11–16–18–18–18	16.2
1–7 days	3–3–3–4–4	3.4
1–7 days	3–3–4–4	3.5
Adult males	8–10–18–24	15.0
Adult females	11–14–17–20	15.5
Mean for 1–7 day fish		6.6

The comparison of mean survival does not give a true picture of the toxicity of endrin, however. It was mentioned earlier that most deaths occurred at the beginning of the test period. This is es-

TABLE 7.—*Survival of guppies in 0.5, 0.4, 0.25 and 0.1 p.p.b. of endrin*

Initial age	Number of days each fish survived	Mean survival in days**
0.5 p.p.b. concentration of endrin:		
1–7 days	4–4–4–5–172*	4.3
Do	4–5–6–6–6	5.4
Do	6–6–7–8–8–9–9–9	7.8
Do	5–6–7–7–8–9–9–9–10	7.8
Do	5–5–6–6–7–7–7–11–14–28	9.6
Do	13–17–20–122*–122*–122*–122*–122*–122*–122*	16.6
Do	8–11–12–13–18–24–24–25–41–41	21.7
Do	25–45–122*–122*–122*–122*–122*–122*–122*–122*.	35.0
Do	5–8–11–82–101	41.4
Do	5–105–106♂–120♂–193♂	105.8
Adult males	25–26–24–154*	25.0
Adult females	31–39–40–44–154*–154*	38.5
Mean for 1–7 day fish		25.5
0.4 p.p.b. concentration of endrin:		
1–7 days	6–10–15–30–32–32–137*–137*–137*.	20.8
Do	7–10–26–42–92–129♀–137*♀	51.0
Do	62–83–87–101*♀–101*♀–101*♀–101*♂–101*♂–101*♂–101*♂.	77.3
Mean		49.7
0.25 p.p.b. concentration of endrin:		
1–7 days	5–80–146♂–163*♂–175*♂	77.0
Do	55–61–163♀–196*♂196*♂	93.0
Do	12–44–45–60–64–70–81–83–97–122*.	61.8
Mean		77.3
0.1 p.p.b. concentration of endrin:		
1–7 days	108–102*–122*–122*–122*–122*–122*–122*–122*.	108
Do	41–70–132♀–188♀–196*♂	108
Do	110♀–118♀–169♀–196*♂	132.3
Mean		116.1
0 p.p.b. concentration of endrin:		
1–7 days	12–70♀–159♀–191♂–196*♂	108
Do	168♀–170♀–177♂–134♂	162.8
Mean		135.4

*Fish were killed at the end of the test.
**Fish which were living at the end of the test are not included in the calculation of mean survival.

pecially noticeable in 0.5 p.p.b. of endrin. An inspection of the individual survival times in table 7 reveals that, in general, fish which survived 30 days were likely to survive much longer. This pattern would be even more definite if the disease had not been present. A discussion of the cumulative effects of endrin will be found in the section beginning on page 29.

Such evidence, plus data on sublethal effects presented in a later chapter, indicate that the threshold falls in a rather narrow range of concentrations near 0.5 p.p.b. endrin. Above this threshold the fish die, and below it the fish live with few physical effects.

The data are too scanty to draw any conclusions concerning a sexual difference in tolerance. On the basis of a cursory examination of the tables, it seems that there is no appreciable difference between the sexes.

The only data on reproduction were obtained from the adults used in the tests. Some of the adult females were pregnant when placed in the test solutions, but of six females which bore young, only one bore a second litter. This female was in 0.5 p.p.b. of endrin. There were six in the first litter, all normal in appearance. In the second litter, 6 of the 12 had large yolk sacs and were probably premature. However, the rate of survival of these young was about the same as for normal ones. The young born from the other five females (two in 0.75 p.p.b. and three in 0.5 p.p.b.) had survival rates similar to normal young also. (No adult females were put into the two lower concentrations.) Whether the effect of the endrin is on the egg or on the sperm is not known. Males frequently attempted to copulate with the females. Of the females which grew to sexual maturity in concentrations of endrin, most contained well-developed eggs when they died. (Death presumably was due to the disease.)

Fish were not measured during the tests, so their growth rates are not known. However, the two highest concentrations definitely retarded or stopped growth. Several fish in 0.5 p.p.b. grew very little. One was 126 days old when killed and measured only 8 mm. standard length, this size being no larger than that of many normal guppies at birth. Since I have noted similar stunting in the past under normal aquarium conditions, this may not have been caused by endrin.

The symptoms of poisoning were very

similar to those described for bluntnose minnows, with one notable exception. Guppies went to the bottom, whereas bluntnose minnows went to the surface. This was unusual, because bluntnose minnows are bottom feeders and guppies are surface feeders.

No data were obtained on increased resistance in offspring of fish chronically exposed to endrin. I planned to run tests on offspring of fish raised in endrin to determine whether there was increased tolerance, but unfortunately no young were obtained for these tests. The only available data on this aspect of the subject come from observations of fishes put into test solutions. Without exception, the fish that survived for long periods in 0.5 p.p.b. endrin went through a period of poisoning with symptoms like those described on page 12, for the females in TB–7. Like the female bluntnose, the guppies returned to normal appearance and behavior after a period of time. Hyperactivity and slight hypersensitivity were the only abnormal types of behavior noticed. If after the initial period endrin's effect is all or none, as I have postulated earlier, then one would not expect tolerance to increase to any great extent.

ACUTE-TOXICITY TESTS ON BLUNTNOSE MINNOWS

Objective

The objective of this part of the investigation was to determine whether there were significant differences between the various size groups in their acute tolerances to endrin.

Methods and equipment

Continuous-flow tests were used to determine the endrin-toxicity values for various size groups of bluntnose minnows. The necessity of using a continuous-flow system as opposed to a static or nonflowing system is often questioned. Henderson and Tarzwell (1957) suggest a static test, probably because it is quicker and easier. When testing volatile substances there is a loss due to evaporation. If aeration is necessary (as it often is if dissolved oxygen is to be maintained at 5 p.p.m. or higher) the loss may become appreciable. When aeration is needed, the above-mentioned authors suggest a slow stream of pure oxygen, but the use of oxygen is very bothersome. Other problems arising in static testing are the accumulation of excretory products, especially carbon dioxide, and the growth of bacteria. Continuous-flow systems hold these factors to a constant minimum, and while these factors are not eliminated in absolute terms, they are eliminated as variables. If aeration is necessary, the continuous-flow system has a distinct advantage.

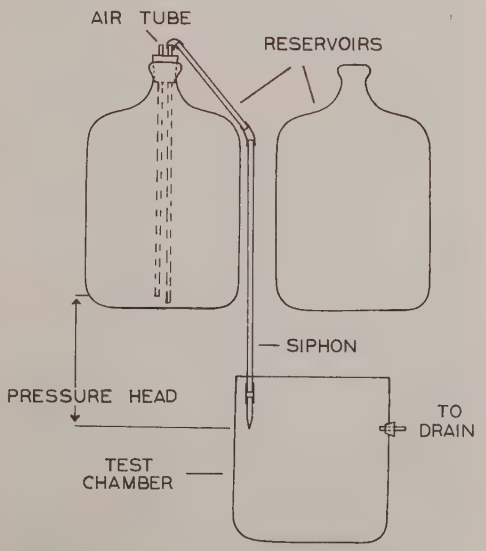

FIGURE 4.—Diagram of continuous-flow system used for acute testing.

Sometimes the amount of a toxic substance absorbed by the test animals in static tests becomes significant, and the continuous flow will offset this effect also. In this test I suspected that the differences between size groups would be slight, if they occured at all. The continuous-flow system was used, therefore, to minimize the lowering of the endrin concentration by absorption.

The test chambers were 5-gallon glass jugs cut off at a height of 12 inches. An overflow hole was drilled 6 inches from the bottom; the capacity at overflow level was approximately 9 liters. These test chambers were placed in a large water bath which was maintained at 22.2° C., plus or minus 1° C. These chambers were supplied from a 5-gallon glass jug reservoir placed on a rack above them. Water was siphoned from the reservoir, and the flow was controlled by means of a small glass jet. Constant pressure was maintained in the siphon by stoppering the reservoir and running the air tube to the bottom of the jug. In this way the pressure on the siphon was a head of water equal to the vertical distance from the end of the air tube to the end of the siphon. Pressure was independent of the water level so long as the level was above the end of the air tube. This system gave a very constant pressure as the jug emptied. All water lines were made of glass, with flexible joints made of tygon tubing. When the flow was 10 ml. per minute, one 5-gallon jug held a 32-hour supply.

There were two jugs for each test chamber, the siphon and air tube being transferred from one to the other. This made it possible to fill one jug 24 hours in advance of its use, which was sufficient time for the water to warm to room temperature and for the free chlorine to escape. Each test chamber was aerated at a uniform rate with a glass air jet. This aeration also ensured adequate mixing. Five test chambers were used, one as a control and four as test chambers.

A stock solution of endrin in alcohol was made for each test chamber, using the initial stock solution of 0.1 mg. of endrin per milliliter of 95-percent alcohol. Additional alcohol was added so that equal volumes of the stock solutions added to each reservoir would give the desired concentration. This volume was 0.00001 ml. of stock solution per milliliter of water. This gave a uniform concentration of alcohol in water in each chamber. The measured amount of solution was placed on a glass stirring rod and lowered into the reservoir. The same volume of pure 95-percent ethyl alcohol was added to the control. Five fish were assigned to each testing chamber by the use of a table of random numbers.

The tests followed the basic routine described by Henderson and Tarzwell (1957). Fish were held for at least 2 weeks at 22.2° C. before testing. They were not fed for 48 hours prior to testing nor during the test. Alkalinity, pH, dissolved oxygen, and free carbon dioxide were measured at the beginning of the test, again when the fish began to die, and finally at the end of the test. The 96-hour $TL_{\bar{m}}$ values were determined by straight-line graphic interpolation. The concentration of endrin was plotted on a log scale and percent survival on a linear scale. A line was drawn between the two points bracketing the 50-percent survival concentration, and the point on the concentration scale at which this line crossed the 50-percent survival level was read as the 96-hour $TL_{\bar{m}}$ value. Percent survival was calculated as the percent living in each chamber after exactly 96 hours. In all cases fish were clearly alive or dead, and there was no problem with borderline cases.

The chemical properties of the water are listed in tables 1 and 8. All determi-

TABLE 8.—*Chemical characteristics of the water in the acute-toxicity test chambers*

[All values, except pH, are expressed in p.p.m. These values are based on 27 determinations]

Characteristic	Range	Mean
Dissolved oxygen	6.4–9.1	7.5
Free carbon dioxide	1.0–0	.5
Phenolphthalein alkalinity	0–14.0	4.1
Methyl orange alkalinity	11.0–32.0	23.2
Total alkalinity	17.0–33.0	27.3
pH	*7.5–9.0	8.4

*The high pH was present only for the first few minutes of the test until aeration reduced it.

nations were made by use of techniques listed in Welch (1948). The unmodified Winkler method was used for dissolved oxygen, and the pH determinations were made colorimetrically as described earlier.

All fish used were collected from the same pool in Rocky Fork Creek as were the animals used in the chronic-toxicity study. With few exceptions, all fish were heavily parasitized by tapeworms (Plerocercoids of *Proteocephalus*) in the mesenteries. These parasites did not noticeably affect the fish, all of which appeared very healthy. On four occasions large numbers of fish (200 to 500) were brought into the laboratory, and each time not more than 10 died during the acclimation period. These died within the first few days, probably as a result of injury when captured. No deaths occurred in the control chamber during the testing. The fish were fed ground dog-chow checkers during the acclimation period before testing. Only three size groups were available, with mean sizes of 29.8, 40.6, and 59.8 mm. standard length; these are designated size groups 1, 2, and 3 in table 9. Fish were preserved in 10-percent formalin and were measured to the nearest millimeter, standard length.

Results and discussion

Table 9 summarizes the data and analysis of variance for the acute tests (Snedecor, 1946). Appendix 1 lists the raw data from the tests. Each 96-hour $TL_{\bar{m}}$ value

TABLE 9.—*Data and analysis of variance for acute-toxicity tests on bluntnose minnows*

A. 96 hour $TL_{\bar{m}}$ values

[Values expressed in p.p.b. endrin in water]

	Size group 1 (29.8 mm. mean S.L.)	Size group 2 (40.6 mm. mean S.L.)	Size group 3 (59.8 mm. mean S.L.)	Sum	Mean
Test 1	.26	.35	.51	1.12	.37
Test 2	.28	.24	.43	.95	.32
Test 3	.28	.28	.46	1.02	.34
Sum	.82	.87	1.40	3.09	
Mean	.27	.29	.47		.34

B. Summary table for analysis of variance

Source	Degrees of freedom	Sum of squares	Mean square
Total	8	0.0786	
Group	2	.0689	0.0345
Test	2	.0049	.0025
Discrepancy	4	.0048	.0012

F value for groups=28.75.
F_{01} value with 2 and 4 degrees freedom=18.00.

is based on five fish in each of four concentrations. Eight preliminary tests were run before a suitable range of concentrations was found.

The F value for between groups exceeds the value of F at the 0.01-percent level, with two and four degrees of freedom, indicating a probable difference in the tolerance of various sizes. This difference, while detectable, is not large enough to be of importance in setting safe levels for natural bodies of water.

No statistical test is available to determine the effect of acclimation period on the toxicity in this case. An inspection of table 9 indicates that the variation within any group is not nearly as great as between certain groups. As an example, the first $TL_{\bar{m}}$ value listed under size group 3 is for fish held 14 days before testing. The third value in the same group is for fish held 57 days before testing. Both groups were from the same collection of January 1, 1960. Reference to appendix table 1, tests S–11 and S–17, will show that the mean sizes of these two groups were 63.4

and 58.9 mm., standard length. The difference in $TL_{\bar{m}}$ values could be due in part to size differences. Since the maximum variation of $TL_{\bar{m}}$ values within any group is not more than 0.09 p.p.b., the length of the acclimation period beyond 14 days (recommended by Henderson and Tarzwell, 1957) does not introduce variation of practical significance.

The effect of temperature on toxicity was investigated by Iyatomi et al. (1958), who found that a decrease of 20° C., from 27° C. to 7° C., caused the tolerance of 7- to 8-centimeter carp to increase by a factor of 28. It is doubtful whether this difference would be as great in longer exposures. The effect of temperature should be investigated further.

From table 4 and appendix table 1 it is clear that the fish used in the chronic-toxicity test were more tolerant to endrin than those used in the acute-toxicity tests. At first it seemed most probable that the lower tolerances of the fish in the acute tests were caused by physiological changes in the fish as a result of the colder weather, since all fish used in the acute tests were collected on December 6, 1959, and January 1, 1960. If this were the case, one would expect the acute $TL_{\bar{m}}$ values to change as the fish were held for longer periods. This does not appear to be the case, as mentioned above.

There are three other possible causes for this difference: (1) The fish used in the chronic-toxicity tests were not parasitized and may have been in better physical condition. (2) The fish used in the chronic-toxicity tests were from smaller populations in less crowded conditions. The stream population was extraordinarily large when the fish used in the acute-toxicity tests were collected. Langlois (1951) reported that bluegills living in crowded conditions were less hardy. (3) The toxic effect of endrin may be affected by the reproductive physiology. Whatever the cause of the reduced tolerance, the effect should have been the same for all groups, and therefore the difference found between size groups should be a true one.

Since bluntnose minnows cannot be sexed by external characters, except during the breeding season, any sexual differences in the tolerance to endrin must be obtained from the acute toxicity tests. Sexually mature fish were used in five acute-toxicity tests, and the mean survival time of both the 56 females and the 44 males used in these tests was 3.2 days. Treon, Cleveland, and Cappel (1955) and others found that in mammals there is a sexual difference in tolerance to endrin.

EFFECT OF ENDRIN ON OXYGEN CONSUMPTION

Objective

The objective of this part of the investigation was to determine whether endrin has any effect on the rate of oxygen consumption. The large increase in the ventilation rate indicated that the fish might be using more oxygen.

Methods and equipment

A system similar to the one described by Keys (1930) was modified and used for these oxygen-consumption tests. The basic plan was to measure the difference in the dissolved oxygen in the effluent of two closed chambers, one containing a fish and one without a fish. A reservoir and constant-head chamber were used as a water supply for the oxygen tests (fig. 5). The water was aerated with compressed air in the constant-head chamber. Two 1-quart glass jars with stoppers served as test chambers. They were submerged in a water bath which was main-

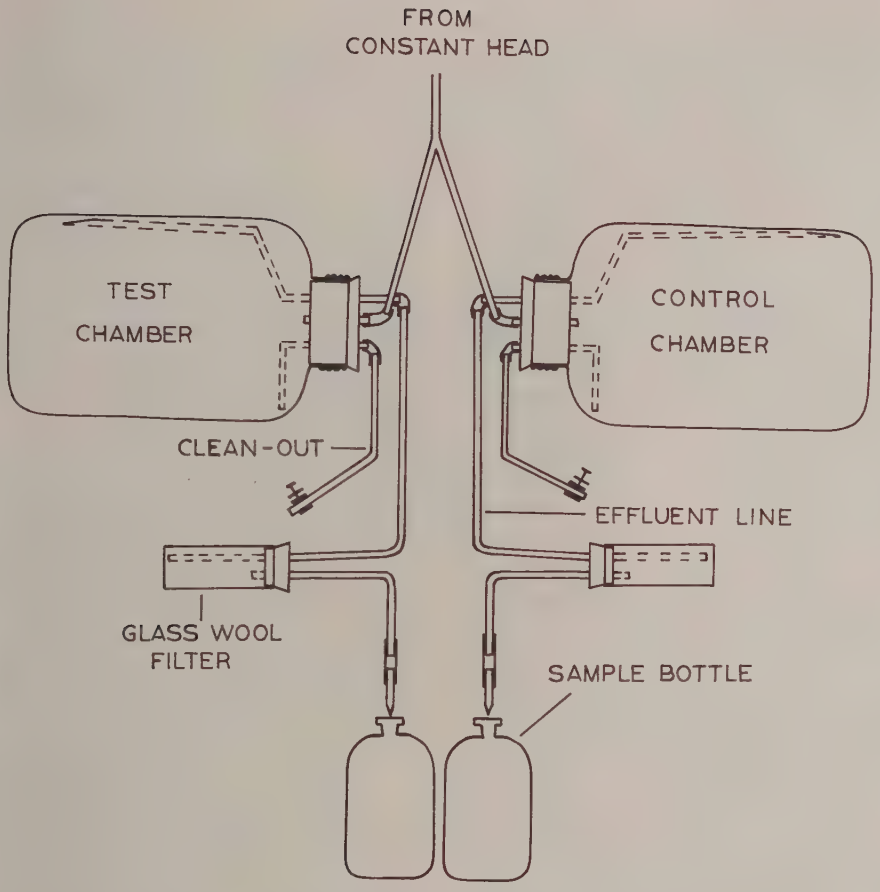

FIGURE 5.—Diagram of apparatus used to measure oxygen consumption.

tained at 24° C. by a commercial aquarium heater. Temperature stayed within the range of 23° to 24° C. Each jar was placed on its side, and water was introduced into the stoppered end through a siphon and removed by another syphon leading from the top of the closed end. The test-chamber effluent was filtered through a glass-wool filter which was cleaned every 2 days to prevent bacterial growths. Flow rates were regulated by means of small glass jets, and were measured with a 10-ml.-capacity graduated cylinder. A flow rate of 6 ml. or 12 ml. a minute was used, depending on the size of the fish being tested. If a flow rate of 12 ml. a minute was used, the flow was measured for 30 seconds three consecutive times and the average of the three readings was used. If a flow of 6 ml. a minute was used, the flow was measured one time for a period of 1 minute. Measurements of flow rate were made immediately after the sample for oxygen determination was taken. The dissolved-oxygen concentration of the effluent from the test chamber was approximately 4 p.p.m. On three occasions it went down to 3 p.p.m.

Sample bottles, 125-ml. capacity, were placed under the glass jets at such a level that when the bottle was overflowing only $\frac{1}{16}$ inch to $\frac{1}{32}$ inch of the glass jet was be-

neath the surface of the water in the bottle. In this way the sample bottles could be filled and left under the jets all the time without reducing the flow or admitting oxygen to the water. Sample bottles were left under the water jets for a minimum of 4 hours before the dissolved oxygen was determined. This method was also advantageous in that the sample could be removed before my presence disturbed the fish and caused its oxygen consumption to increase. The 125-ml. sample was representative of the preceeding 10 minutes, or 20 minutes, depending on the flow rate, and therefore small increases of oxygen consumption for short periods did not disrupt the determinations. Methylene blue was used several times to determine whether the water in the sample bottles was being completely mixed by the water stream. It was found that the stream of water from the jet went to the bottom of the sample bottle and then moved outward and upward. By the same method it was found that good mixing was also taking place in the test chambers.

The Winkler method, with a few minor changes, was used for dissolved-oxygen determinations. All volumes of the solutions used were reduced by half, and N/100 sodium thiosulfate was used instead of N/40, in order to obtain greater accuracy. One hundred milliliters of the sample were measured with a volumetric pipette and titrated. Since oxygen consumption was based on the difference between the two sample concentrations, no correction for loss of oxygen due to addition of reagents was necessary.

In the first tests, the fish were fed ground dog-chow checkers daily through a T-joint in the water line. In later tests, feeding was discontinued because it was felt that it introduced too much variation into the consumption rate. This was true even in readings taken 24 hours after the fish were fed. See figures 6 through 12.

The fish were weighed in water on a beam balance to the nearest tenth of a gram before the test and were measured and sexed after the test. All fish used for oxygen-consumption tests were collected from the same location as those used in the other testing. They were held at 18° to 20° C. before being tested.

After a fish had been weighed, it was placed in one test chamber and left for a few hours, usually overnight. Then serial measurements of its oxygen consumption were begun. Measurements were made every 24 hours in tests O–1, O–2, O–4, and O–5, and at about 12 hour intervals in tests O–6, O–7, and O–8. Disturbances were at a minimum in the basement where the tests were conducted. There were few vibrations, because the floor was concrete. The actual formula used for determining oxygen consumption was as follows:

$$\frac{C \times 0.8 \times F \times 0.698}{W} = \text{ml. of oxygen per gram per hour.}$$

C is the difference in titration values between test and control samples.

0.8 is a factor for converting titration values to mg.

F is the flow rate in liters per hour.

0.698 is the factor to convert milligrams of oxygen to milliliters of oxygen under standard conditions.

W is the weight of the fish in grams.

Results

Table 10 lists the mean consumption rates for each fish tested. In the first four tests, the fish were held in the test chamber for 5 to 15 days in an attempt to get a constant consumption reading. There was little change in consumption after the first few hours, however, so in later tests the period of time before endrin was introduced was shortened.

Although with one exception the mean oxygen consumption is higher in the presence of endrin than under normal condi-

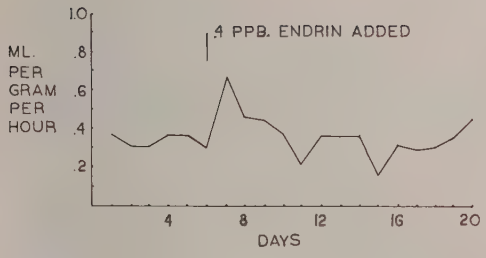

FIGURE 6.—Test O–1. Oxygen consumption of a 0.7-gram female bluntnose minnow, 38 mm. standard length. Fish was fed in the evening and consumption was determined at 7 a.m.

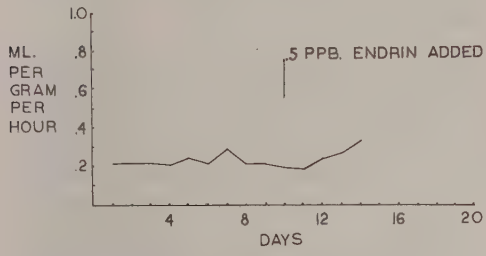

FIGURE 7.—Test O–2. Oxygen consumption of a 4.2-gram male bluntnose minnow, 70 mm. standard length. Fish was fed in the evening and consumption was determined at 7 a.m.

tions, the difference is not over 0.06 ml. per gram per hour in any case. Usually the difference between means is less than the daily variation.

Figures 6 through 12 represent graphically the oxygen consumption for all fish tested. In test O–8, figure 12, oxygen consumption was measured every 4 hours for 16 hours after endrin was added, but no variation was found.

Discussion

Harvey and Brown (1951) report that compounds related to endrin (aldrin and dieldrin) cause a fivefold increase in oxygen consumption when injected into the cockroach. They found a latent period of 200 to 300 minutes before oxygen consumption reached the peak and then tapered off until death. No such increase was noted in the bluntnose minnows; such

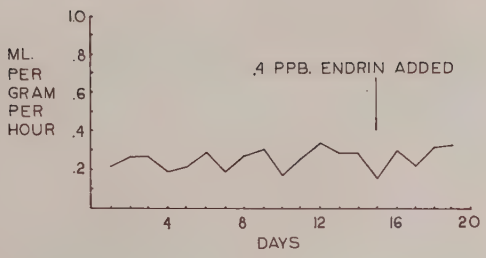

FIGURE 8.—Test O–4. Oxygen consumption of a 5-gram male bluntnose minnow, 73 mm. standard length. Fish was fed approximately 24 hours before consumption was determined at 7 a.m.

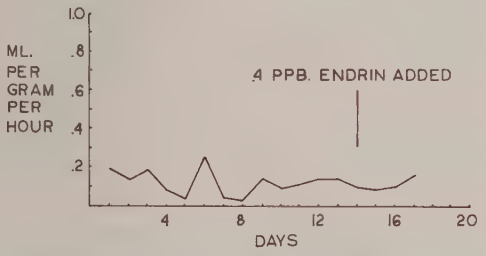

FIGURE 9.—Test O–5. Oxygen consumption of a 2-gram female bluntnose minnow, 56 mm. standard length. Fish was not fed. Consumption was determined at 7 a.m.

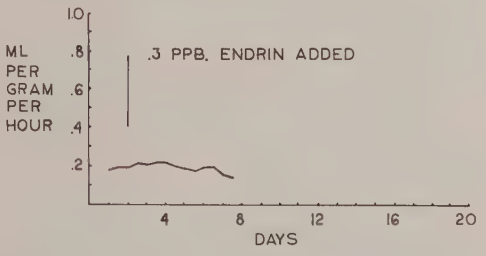

FIGURE 10.—Test O–6. Oxygen consumption of a 2.6-gram female bluntnose minnow, 60 mm. standard length. Fish was not fed. Consumption was determined at 7 a.m. and 6 p.m.

increases as occurred were 25 percent or less above normal. Fry, in The Physiology of Fishes (Brown, 1957), gives data on goldfish which show a fourfold increase in oxygen consumption due to activity. The slight increase found here in fish exposed to endrin would be most logically attrib-

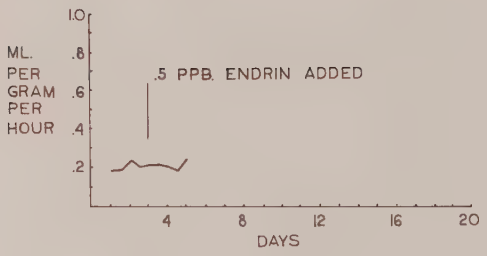

FIGURE 11.—Test O–7. Oxygen consumption of a 3-gram male bluntnose minnow, 61 mm. standard length. Fish was not fed. Consumption was determined at 7 a.m. and 6 p.m.

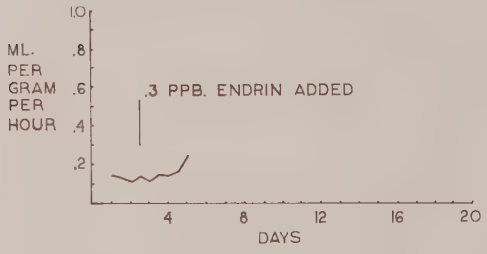

FIGURE 12.—Test O–8. Oxygen consumption of a 3-gram male bluntnose minnow, 61 mm. standard length. Fish was not fed. Consumption was determined at 7 a.m. and 6 p.m.

uted to the increased movement due to tremors and convulsions.

The insignificant increase in oxygen consumption is surprising in view of the great increase in ventilation rate. No counts of ventilation rates were feasible on small fish with such high rates, but it appeared that the fish were ventilating at a maximum rate. Since the ventilation rate increased greatly, while the rate of oxygen consumption did not increase significantly, and since the ability of the fish to work was not affected, it seems that the apparent respiratory distress must be due to a stimulus other than a deficiency of oxygen in the tissues. The effect could be attributed to accumulation of metabolites.

TABLE 10.—*Mean oxygen-consumption rates of bluntnose minnows*

Tests O–1 and O–2: Fed 12 hours before readings were taken.
Test O–4: Fed 24 hours before readings were taken.
Test O–5: No feeding for 12 days before endrin was added.
Test O–6: No feeding, killed after 6 days in endrin.
Test O–7: No feeding for 1 day before endrin was added.
Test O–8: No feeding for 9 days before endrin was added.

Test No.	Weight in grams	Standard length in mm.	Sex	Normal consumption ml/g/hr.	Consumption in endrin ml/g/hr.
O–1	0.7	38	F	0.35	0.37
O–2	4.2	70	M	.22	.25
O–4	5.0	73	M	.23	.28
O–5	2.0	56	F	.11	.11
O–6	2.6	60	M	.18	.19
O–7	3.0	61	M	.20	.21
O–8	3.0	61	M	.13	.16

EFFECT OF ENDRIN ON SWIMMING OF BLUNTNOSE MINNOWS

Objective and method

The object of this experiment was to determine whether endrin reduces the ability of fish to carry on muscular exercise. The fish were forced to swim against a current in a current tray (Mount, 1959). The tray was 2 feet wide by 4 feet long by 6 inches deep, with a divider in the center. A paddle wheel powered by a pump motor propelled the water around the tray. The tray was modified slightly in that the corners were rounded, thereby increasing the current to approximately 9 inches per second. Eddies and slow areas were eliminated by placing bricks on the bottom under them, so that the fish could not rest in currentless water.

Test fish were specimens from the highest concentrations of the acute-toxicity tests in which four or five of the fish had survived. Control fish were taken from the control chamber. Both groups of fish had not been fed for 6 days before testing. All testing on both sexes was at 22.2° C. The test fish were distinguished from the control fish by clipping opposite pelvic fins on each group. The paddle wheel was started and a periodic check was made to remove any dead or exhausted fish.

Results

The first two tests were run on fish 30 to 43 mm. standard length, which had been in a concentration of 0.2 p.p.b. endrin for 4 days. In both tests the test fish were tremulous. In the first test one fish died after 3.5 hours. The other four test fish, the five controls, and all five test fish and the five controls in the second test swam against the current for 48 hours, at the end of which the test was discontinued.

In the third test, four fish from the test chamber containing 0.4 p.p.b. endrin were used. They were at the surface and in the latter part of the convulsion stage when the test was begun. They ranged in size from 49 to 75 mm. standard length. Five hours after the current was started one died, and after 8 hours another died. The other two test fish and the five controls were alive and normal after 48 hours.

The last test was run on five fish from the test chamber containing 0.4 p.p.b. endrin. The fish were in the early stages of convulsions. One died after 24 hours, but the remaining four test fish and the controls lived for 48 hours. These fish ranged in size from 56 to 59 mm. standard length.

Discussion

It is my opinion that the three fish which died in the last two test runs would have died if they had been in quiet water. One fish which died in the first test may have died from exhaustion or may have been injured while being handled. In tests 3 and 4, the fish stopped having convulsions as soon as the current was started. In test 3 I turned the current off momentarily after about 30 minutes, and they began having convulsions again.

The only conclusion which can be drawn is that in acute poisoning endrin has little effect on the ability of fish to swim against a current. However, it is possible that if the fish could have been tested in a much swifter current in which even normal animals became exhausted, the test fish would have become exhausted first.

PORT OF ENTRY INTO AND DISTRIBUTION OF ENDRIN IN THE FISH'S BODY

Objective

The extreme toxicity of endrin to fish causes one to wonder what the specific action of endrin might be. The symptoms appearing during the period of poisoning indicate that the effect is mediated through the nervous system. In an effort to gain insight into this problem, an experiment was conducted to determine the point of entry and the movement of endrin through the body.

Methods

Davidow and Sabatino (1954) describe a method of steam distillation by which very low concentrations of endrin were recoverable. When a solution or material containing an endrin residue is boiled in an aqueous solution or slurry, and the steam is condensed and collected, most of the endrin is found in the first 200 ml. of distillate.

With slight modification, this method was employed successfully. A 1,500-ml. Erlenmeyer flask was connected to a Liebig condenser. The material or solution to be extracted was placed in the flask. A fraction of the volume was collected as distillate and the bioassay was run in the distillate. After experimenting with solutions of known concentrations, it was found that collection of 250 ml. of distillate, regardless of the original volume, gave satisfactory recovery. By collecting 250 ml. of distillate from 1,000 ml. of solution known to contain 0.5 p.p.b. endrin, it was possible to get a concentration of 1.5 p.p.b. or more in the distillate, as deter-

mined by a fish bioassay. This represents a recovery of 75 percent or more.

Several bluntnose minnows (with a total weight of 8.1 grams) which had died in 0.5 p.p.b. endrin in 5 days were cut into small pieces and put into a flask with 500 ml. of water. Two hundred and fifty milliliters of distillate were collected, and a small bluntnose minnow was put into this solution. At the same time, another small bluntnose minnow was placed in 250 ml. of a solution containing a known concentration of 2.0 p.p.b. endrin. The fish in the distillate died in 11 hours and the one in the known concentration in 15 hours, which indicated a concentration of over 2.0 p.p.b. in the distillate. Another fish was added to the distillate, and it died in 10 hours. Other distillations were made with similar results. Fish not exposed to endrin were also distilled, but no deaths occurred when the distillates were assayed. From this evidence it was concluded that the technique was reliable. It was also more sensitive than the best chemical test, which is only accurate to 5 p.p.b. Bioassay also has the advantage of being a test of toxicity, which would therefore be positive even if the endrin were slightly altered chemically within the fish or by the distillation. A chemical test might give negative results if the endrin were chemically altered (Klein et al., 1956).

No attempt was made to determine whether the endrin had been chemically altered. One purpose of this testing was to estimate how much endrin accumulates in the body, as an index to the potential danger of such fish in the food chains and to man. So long as the compound is toxic, it makes little difference what its specific form may be. Some insecticides are changed into different compounds. For example, aldrin is changed to dieldrin and stored in the fat (Bann et al., 1956). Kunze and Laug (1953) found that endrin "of a different chemical nature" occurred in the kidneys.

For the study of endrin's entry and distribution within the fish, large carp of approximately 40 cm. standard length were used. They were "snagged" from the Scioto River at Columbus with treble hooks. Only those not seriously injured were used for testing. They were maintained for 2 or 3 days before being put into endrin. In the test, a 30-gallon porcelain tub was used for each fish. Twice daily the tub was partially drained and 10 gallons of fresh solution were added. The temperature was approximately 20° C.; no attempt was made to control it. The test numbers in table 11 represent the following exposure times to endrin:

	Concentration in p.p.b.	*Exposure time in days*
Test 1	0.0	0.0
Test 2	10.0	2.5
Test 3	5.0	4.0
Test 4	5.0	5.0
Test 5	2.5	28.0

Test 1 served as a control to establish that no toxic substances other than endrin were carried over into the distillate. This fish was killed and distilled immediately after being captured. The fish in tests 2, 3, and 4 died; the fish in tests 1 and 5 were killed.

Immediately after death the fish were opened. The various organs were removed, weighed in water, mascerated, and placed one at a time in the flask. Five hundred milliliters of tap water were added to the flask and distilled until 250 ml. of distillate had been collected. The distillate was clear but had a smell of fish oil which disappeared after 5 to 10 hours at room temperature. All distillates were left at room temperature for approximately 12 hours before being assayed.

Each distillate was diluted with tap water so that a 250-ml. aliquot represented the extract from 10 grams of tissue. Thus

if there were 100 grams of tissue, 2,250 ml. of tap water would be added to the 250 ml. of distillate, the solution mixed, and 250 ml. aliquot used for the bioassay. In this way errors in weighing tissue were minimized, and since each 250 ml. sample represented the extract from 10 grams of tissue, the times of exposure to the extract until convulsions set in (and therefore the concentrations) could be compared directly. Ten grams was selected as the basic weight because this was the usual minimum weight of the smaller organs.

The assay was carried out in 1-quart glass jars at room temperature with very gentle aeration, for a 48-hour period. Two hundred and fifty milliliters of various known concentrations were placed in similar containers for comparison. In the fourth test, enough endrin was added to each distillate and to the known concentrations to make a 0.5 p.p.b. solution. In the fifth test, enough was added to make a 1.0 p.p.b. solution. Endrin was added for two reasons: (1) It speeded the test but did not affect the results, since concentrations were determined by comparison. (2) It minimized the effect of individual variability which is less significant at higher concentrations. All fish used in the tests were bluntnose minnows, 20 to 30 mm. standard length, from the lot collected on January 1, 1960, for use in the acute-toxicity tests. As shown by the acute-toxicity testing (appendix table 1), fish in this size-range showed little variability in tolerance. Daphnia and insect larvae were not sensitive enough for assaying the concentrations. One fish was placed in each container for the test.

The onset of convulsions proved to be the most satisfactory end point. The time from the beginning of exposure until the onset of convulsions was quite uniform and predictable within a known concentration (see table 11), whereas that from the beginning of exposure until death was found to be much more variable. The latter took longer for some fish in 10 p.p.b. than for others in 5 p.p.b. Davidow and Schwartzman (1955) found the same variation in their bioassays with goldfish, and they too used convulsions as the end point. Tarzwell and Henderson (1956) used loss of equilibrium as an end point. Klein et al. (1956) used both convulsions and loss of equilibrium as end points. In this investigation, loss of equilibrium was more variable than onset of convulsions.

Results

Table 11 summarizes the results of the bioassays on the corp organs. In tests 2, 3, and 4, heart-spleen-blood (combined), digestive tract, kidney, and liver ranked highest in endrin concentrations. In addition, in test 5 the brain contained a measurable amount of endrin, as did the fat. In test 5, blood was tested separately and contained measurable amounts of endrin, but heart-spleen ranked higher.

TABLE 11.—*Bioassay of extracts of carp tissues using bluntnose minnows*

[Tests were conducted for a 48-hour period in the extract from 10 grams of tissue, except as noted. "Neg." indicates that no convulsion occurred in 48 hours]

	Hours until the onset of convulsions of bluntnose minnows				
	Test 1	Test 2	Test 3	Test 4 [1]	Test 5 [2]
Known concentration of endrin:					
15 p.p.b.		2.75			
10 p.p.b.		3.0	4.5	3.0	3.25
5 p.p.b.		4.0	6.5	4.0	6.25
2.5 p.p.b.			7.5	7.0	10.75
Carp tissue extracts:					
Blood					8.25
Brain [3]	neg.	neg.	neg.	[4] neg.	[5] 6.25
Fat	neg.	10.3	neg.	neg.	8.75
Gills	neg.	neg.	neg.	neg.	12.25
Heart, spleen, blood	neg.	5.25	25.5	[6] 19.0	[7] 3.25
Digestive tract	neg.	7.25	13.0	40.0	10.75
Kidney	neg.	6.25	48.0	19.0	[8] 4.00
Liver	neg.	7.0	48.0	19.0	7.75
Muscle	neg.	9.0	neg.	neg.	neg.
Ovary	neg.		neg.	neg.	neg.
Testes		10.25			

[1] 0.5 p.p.b. endrin added.
[2] 1 p.p.b. endrin added.
[3] Includes some fat in tests 1, 2, 3, and 4.
[4] 8.5 g. tissue.
[5] 3.2 g. tissue.
[6] 9.5 g. tissue.
[7] 6.2 g. tissue.
[8] 6.5 g. tissue.

In previous tests, blood was included with these two tissues so that the total weight was approximately 15 grams; therefore, the distillate had to be diluted by one-half. This would tend to mask out a high concentration in the spleen or heart.

Some error is introduced by the variability of the assay animals, and by the small size of the sample. The four tests do present a consistent picture, however, and for this reason are considered reliable.

Discussion

On the basis of the data in table 11, it may be inferred that endrin is being carried away from the place of entry into the body by the blood. Of the organs assayed, excepting the gills, those having a rich blood supply contained the most endrin. The high concentrations found in the kidney suggest that endrin may be excreted through that organ. Other investigators (previously cited) have found significant damage in the kidney, further suggesting the importance of this organ in the excretion of endrin.

If the kidney is the organ excreting endrin from the body, the high concentration found in the digestive tract presents a puzzling question. The gills gave mostly negative results. (The one positive value may have been due to the endrin added to the distillate.) It seems logical that endrin would have been detected in the gills if it were entering there. It is more probable, in view of the evidence, that the digestive tract was absorbing the endrin. This hypothesis accounts for the negative results of the gill tissue and the positive results of the intestinal tissue.

Since the carp were not fed during the exposure, endrin could not have been taken up from the water swallowed in connection with food ingestion. The statement appears in Brown's Physiology of the Fishes (1957) that "Most of the water is taken in through the semipermeable gill and oral tissues, although some also crosses the less permeable body surface . . . The only other water intake in fresh-water fishes is that accompanying or bound in the food swallowed." There is reason to doubt this statement, at least in the case of some species of fresh-water fish. Both Ellis (1937) and Allee and Frank (1948) have reliable data to the contrary, that fresh-water fish may drink sizable amounts of water. The latter workers showed this to be the case in goldfish. Since the carp is closely related to the goldfish, it is not unreasonable to assume that carp may also drink water, even when not feeding. Allee and Frank give a complete summary of the literature on water intake by fresh-water fish.

Further evidence that the digestive tract may be the port of entry is furnished by Iyatomi et al. (1958) who found that the 2-day larvae of carp are drastically different from 6-day-old fish in their sensitivity to endrin. They found that the 96-hour $TL_{\bar{m}}$ value for carp decreased from 10.7 p.p.m. of endrin at 2 days of age to 0.046 p.p.m. at 6 days of age. This represents a change of toxicity in the order of 250 times. It is quite probable that during this 4-day period the mouth became functional and the larvae began to drink water containing endrin.

It is also possible that endrin is excreted by the intestine and so appeared in the distillate of the digestive tract. Since the intestine was not emptied daily, there could have been an accumulation of excreted endrin in the lumen.

The positive values for the brain in test 5, and for fat in tests 2 and 5, are in agreement with the known storage of chlorinated-hydrocarbon insecticides in those tissues (Sherman and Rosenberg, 1953; Kunze and Laug, 1953; Treon, Cleveland, and Cappel, 1955; and Bann et al., 1956).

Nothing has been said up to this point concerning the absolute concentration

present in the carp tissues. One must be very cautious in estimating concentrations based on bioassay, especially with a small sample. However, Klein et al. (1956) and Davidow and Sabatino (1954) have predicted concentrations on the basis of two goldfish in 250 ml. of distillate. They found the goldfish to give as reliable results as assays using 100 flies.

Perhaps an idea of the order of magnitude can be obtained from the available data. Only in test 5 (table 11) is the time until convulsions in the extract close enough to that in a known solution to warrant an actual estimate of the concentration of the extract. The time until convulsions in heart and spleen extract was the same as in 10 p.p.b. of endrin. If we assume 1 ml. of water to weigh 1 gram, there must have been a total of approximately 2.5 micrograms of endrin present. Since the tissue weight was 6.2 grams, this represents a concentration in the tissue of approximately 400 p.p.b. The concentration in the kidney would be slightly less, as would the concentration in the brain-spinal cord tissue. This represents a level 160 times more concentrated in the tissue than in the water in which the fish was living. The evidence indicates that endrin is actively taken up by some process at some point or points in the body.

It appears that if these estimates are even approximately reliable, there would be little danger to mammals eating exposed fish. These fish, eaten in normal quantities, would contain less than a lethal dose of endrin. The hazard would be further reduced in the case of man, since he normally eats only the muscle tissue, which gave negative results in three out of four tests. Most cooking methods would also evaporate much of the endrin, although Hunt and Bischoff (1960) found that baking fish in foil did not remove DDD.

Muscle from a carp exposed to 2.5 p.p.b. endrin for 4 weeks was fed to two male mice for 3 weeks. Neither mouse showed weight loss or any symptoms of poisoning, indicating that endrin was not present in concentrations high enough to be damaging. Of course, little is known about the effects of very minute amounts consumed by several generations of animals, and it is not safe to draw a conclusion based on this small sample.

In summary, this experiment showed highest concentrations of endrin in the liver, heart-spleen, and kidney. These findings agree with work cited on page 4, in which experiments with rats chronically exposed to endrin revealed that these organs were the ones evidencing tissue damage or storage of endrin. The presence of endrin in the brain and fat in test 5 agrees with the work of other investigators who found that chlorinated hydrocarbons are stored in the brain and fat. However, the cited investigation on mammals did not show any damage or accumulated endrin in the intestine, whereas the carp intestine contained measurable amounts.

MODE OF ACTION OF ENDRIN ON ANIMALS

When a substance is as toxic to an animal as endrin is to fish, the question arises what the specific effect might be. The concentration toxic to fish is usually a few parts per billion, whereas the level toxic to insects is in the range of 100 or more parts per billion. It has been found also by Henderson, Pickering, and Tarzwell (1959) and Iyatomi et al. (1958) that the range of the $TL_{\overline{m}}$ values among species of fishes tested is not over 5 parts per billion. Endrin's toxicity to mammals is also less than its toxicity to fish. Ely et al. (1957) reports 1.5 mg./kg. to be toxic to cattle,

and Treon, Cleveland, and Cappel (1955) determined that 43.4 parts per million of endrin in food was toxic to rats.

Hartley and Brown (1955) reported that endrin had no effect on the cholinesterase level in the American cockroach. Morrison and Brown (1954) found that compounds related to endrin had no effect on the cytochrome oxidase level in the same animal. Nelson (1955) states that serum alkaline phosphatase levels were higher in test than in control rats fed 100, 50, 25, 5, and 1 p.p.m. of endrin in their food. (In human beings an increase in the serum alkaline phosphatase levels may indicate liver disturbances.—Hawk, Oser, and Summerson, 1954). All rats were hypersensitive to stimuli, and those fed 100, 50, and 25 p.p.m. endrin in the food had convulsive spasms. Roeder (1948) found that technical chlordane (related to endrin chemically) had no effect on synaptic transmission of impulses in the nerve preparation of *Periplaneta* and caused no neurologic disturbances in mammals.

Harvey and Brown (1951) stated that all insecticides which increase oxygen consumption are known to be nerve poisons. He used chlordane as an example of one which is not a nerve poison and which does not increase oxygen consumption significantly.

A clue to the possible mode of action was found during the testing in this investigation. The symptoms—convulsions and loss of equilibrium—indicate disturbances in the central nervous system. There was always an increased ventilation rate but, as was pointed out earlier, the oxygen consumption did not increase. In many of the young guppies which died in endrin, the skull burst and the brain protruded. This indicated pressure on the central nervous system. Such pressure would also stimulate the respiratory center, produce convulsions, and cause loss of equilibrium.

A condition called papilledema in humans, in which the optic disk swells as a result of pressure in the central nervous system, is used to detect diseases which cause an increase in pressure on the brain in man. The pressure is transmitted along the optic nerve, collapses the blood vessels, and causes the optic disk to swell because of the blocked circulation.

I attempted to find papilledema in fish by fixing and measuring optic disks of normal fish and of fish which had died in 10 p.p.b. endrin. Four fish were placed in 10 p.p.b. endrin and four of similar size were placed in tap water. As the fish in endrin lost equilibrium they were put into A. F. A. fixing solution. When the fourth test fish was fixed, the control fish were fixed also. After 12 hours in the fixative, the eyes were removed and imbedded in paraffin. Serial cross-sections 15 microns thick were made and stained with eosin. Measurements were made with an ocular micrometer.

The results of this experiment are shown in table 12. Two measurements were taken on each eye; the minimum thickness of the two middle sections was measured. No significant difference was found between the treated and the control groups. It is possible that shrinkage due to fixation obliterated any swelling which may have been present.

TABLE 12.—*Optic-disk measurements of the bluntnose minnow*

[Two sections of each disk were measured. Numbers represent ocular-scale divisions in which 1 division equals 6.8 microns]

Normal fish		Fish in 10 p.p.b. endrin	
Left eye	Right eye	Left eye	Right eye
22	28	33	24
20	26	31	25
40	28	30	20
45	27	33	25
24	20	22	24
21	19	21	20
28	25	35	25
31	23	30	25
$\bar{s}$ length of fish=48 mm. standard length		$\bar{s}$ length of fish=46.8 mm. standard length	
$\bar{m}$ thickness of optic disk= 26.7 ocular divisions		$\bar{m}$ thickness of optic disk= 26.4 ocular divisions	

The carp used in the investigation of the entry and distribution of endrin in the body were examined with an ophthalmoscope while convulsions were in progress, but it was very difficult to see into the eye and I could not tell anything about the optic disk. Carp were difficult to hold still, and the examination was unsatisfactory.

When fish showing symptoms of poisoning are placed in pure water, they usually recover rapidly. In one instance, 10 bluntnose minnows were placed into a continuously renewed solution of 0.3 p.p.b. When five fish had died and the other five were having convulsions, the flow was stopped. Two days later approximately half of the 9 gallons in the test chamber were replaced with tap water. These five fish went through the symptoms of endrin poisoning in a reverse order, and at the date of this writing, 3 months later, appear normal and healthy. This type of reaction indicates that endrin does not cause irreversible damage.

The available information concerning the mode of action of endrin on animals does not permit any conclusion to be drawn about the specific effects of endrin. This present investigation furnishes little new information other than that endrin does not appear to increase oxygen consumption, nor does it appear to cause irreversible damage as a result of short exposures.

SUMMARY

Investigations of the chronic toxicity of endrin to bluntnose minnows, *Pimephales notatus*, and guppies, *Lebistes reticulatus*, were conducted for periods of 10 and 6 months, respectively. Continuously renewed solutions were employed.

Approximately 35 percent of the bluntnose minnows survived for extended periods in 0.5 p.p.b. endrin in water, and approximately 50 percent survived 0.4 p.p.b. All bluntnose minnows placed in 0.1 p.p.b. lived for extended periods; all died in 0.75 and 0.9 p.p.b. within a 29-day period.

Symptoms of endrin poisoning in the bluntnose minnows were characteristic of disturbances in the central nervous system: hyperactivity, increased ventilation rate, convulsions, loss of equilibrium, and death. The symptoms lasted for longer periods at lower concentrations. Some fish were in a state of hyperactivity and increased ventilation rate for as long as 2 months and then returned to normal condition while still in endrin. Mean daily growth rates in 0.5, 0.4, 0.1, and 0 p.p.b. endrin were 0.049, 0.047, 0.102 and 0.069 mm., respectively. The toxic levels for chronic exposures were not greatly different from those toxic in acute exposures. Fish in 0.5 and 0.4 p.p.b. exhibited much more activity than those in 0.1 p.p.b. and in the control aquariums. The gonads in both sexes in all test chambers showed increased activity and appeared to be ripening about as rapidly as those of fish taken from the stream. Histological examination showed fatty vacuolation of the liver cells in many of the fish, including control fish. Such vacuolation is thought to be due to the high fat content of the diet fed. No tissue damage was found in the brain, heart, kidney, or spleen.

A chronic-toxicity test on guppies was conducted simultaneously with the bluntnose minnow test. Because of a disease of the kidneys which was apparently unrelated to the presence of endrin, tests on the guppies were discontinued after 6

months. The effects of endrin on the guppies were essentially the same as those on the bluntnose minnows.

Pregnant female guppies, with one exception, never had more than one litter of young after being placed in 0.75 and 0.5 p.p.b. endrin in water. In one case, a second litter of 12 was produced and included 6 young with large yolk sacs, presumably born prematurely. All young born from females in endrin, including the premature ones, survived about as long as guppies born from parents not in endrin. No sexual difference in survival was found. Adults were usually more tolerant than young.

One major difference in behavior was found during the period of poisoning; guppies went to the bottom and bluntnose minnows went to the surface. The normal resting and feeding positions of these species are just the reverse.

In acute-toxicity testing of bluntnose minnows, fish with a mean standard length of 59.8 mm. withstood 0.2 p.p.b. more endrin in 96 hours than did fish of mean standard lengths of 29.8 and 40.6 mm. The fish used in the chronic-toxicity test (collected in March) proved to be more tolerant to endrin than those used in the acute-toxicity tests (collected in the winter). This may have been due to one or more of the following: (1) the stage of the reproductive cycle, (2) parasitism, (3) seasonal temperatures and related physiological conditions, (4) crowded conditions in the stream resulting in less hardy fish.

Endrin had no apparent effect on oxygen consumption of fed and nonfed fish of either sex. Endrin did not seem to affect the ability of fish to swim against a current of water.

Evidence was found which indicates that endrin enters the bodies of carp through the intestines. It probably is carried by the blood to other parts of the body. The liver, intestine, spleen, and kidney of carp contained the highest concentrations of endrin in 2- to 5-day exposures. Blood, fat, and brain tissue also contained measurable amounts after a 28-day exposure. All concentrations in carp tissues were based on a fish bioassay of distillates collected by steam distillation.

The bursting of the skull and subsequent protrusion of the brain of young guppies which died in various concentrations of endrin indicated that endrin may cause pressure in the central nervous system.

CONCLUSIONS

Endrin does not appear to be cumulative in its effect on fish subjected to chronic exposures. There was a narrow range from 0.4 to 0.5 p.p.b. in which fish showed some loss of weight and greatly increased activity. No effect was found below this concentration. Although no reproduction occurred in the bluntnose minnow, the gonadal development indicated that reproduction might have taken place if the test had been continued. Concentrations as low as 0.5 p.p.b. appeared to prevent reproduction in guppies.

It appears that the increase in activity caused by endrin in concentrations of 0.5 and 0.4 p.p.b. might be of greatest significance in natural populations through disruption of spawning behavior and displacement from their normal habitat niche.

On the basis of the data, endrin appears to enter the body through the intestine as a result of the fish's drinking water. Very young fish are resistant to endrin, probably because their mouths are not functional and the endrin does not get into the intestine.

There is evidence of a slight accumulation of endrin in carp tissue during a 28-day exposure. The low concentration found in the muscle tissue would probably not be harmful to human beings eating exposed fish. Cooking would also reduce the concentration.

It appears to be safe to say that long-term endrin would not harm fish seriously so long as a concentration which is not lethal in 96 days is not exceeded. Endrin is extremely toxic to fish, and application rates would have to be very low. However, if endrin will effectively control pests at an application rate which would be safe for fish, its use would be preferable to that of a compound which is fish lethal even at greatly reduced rates.

There is a very serious need for long-term laboratory studies of the effects of many insecticides at all safety levels. For one such study was found in the literature. Investigations are needed to determine which organisms in the aquatic food chains are most sensitive to various pollutants. More work should be done to determine the residual life of these pollutants in the bottom sediments and in the tissues of animals. Until this information is known, the danger involved in continuous use of insecticides cannot be determined. There is little knowledge at the present time to indicate what longer term effects on human beings, either directly or indirectly, from continuous use of insecticides.

Finally, it will be necessary to gain a better understanding of normal fish physiology before the true dangers of insecticides to fish can be properly assessed.

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Appendix—RAW DATA FROM TESTS

APPENDIX TABLE 1.—*Results of acute-toxicity testing on bluntnose minnows*

["Juvenile" indicates fish too young to sex easily; "A" indicates fish living at end of test]

	Standard length (mm.)	Number of days lived
Test S–9:		
0.8 p.p.b. endrin:		
Juvenile	26	1
Do	27	1
Do	30	1
Do	31	1
Do	32	1
0.6 p.p.b. endrin:		
Juvenile	33	1
Do	24	1
Do	33	1
Do	34	1
Do	29	1
0.4 p.p.b. endrin:		
Juvenile	25	2
Do	29	2
Do	30	2
Do	28	3
Do	34	3
0.2 p.p.b. endrin:		
Juvenile	32	3
Do	22	A
Do	28	A
Do	28	A
Do	31	A
Mean size	29.3	
Test S–10:		
0.8 p.p.b. endrin:		
Juvenile	27	1
Do	28	1
Do	34	1
Do	26	2
Do	34	2
0.6 p.p.b. endrin:		
Juvenile	26	1
Do	26	2
Do	27	2
Do	32	2
Do	31	3
0.4 p.p.b. endrin:		
Juvenile	26	3
Do	27	3
Do	28	3
Do	31	3
Do	32	3
0.2 p.p.b. endrin:		
Juvenile	27	A
Do	28	A
Do	30	A
Do	33	A
Do	34	A
Mean size	29.4	
Test S–11:		
0.8 p.p.b. endrin:		
Male	65	2
Do	66	2
Female	60	2
Male	58	2
Do	81	4
0.6 p.p.b. endrin:		
Male	71	2
Do	70	3
Do	64	3
Do	59	4
Do	70	A
0.4 p.p.b. endrin:		
Male	68	A
Do	70	A
Do	60	A
Female	56	A
Male	57	A
0.2 p.p.b. endrin:		
Male	65	A
Do	57	A
Female	59	A
Do	56	A
Do	55	A
Mean size	63.4	

	Standard length (mm.)	Number of days lived
Test S–12:		
0.8 p.p.b. endrin:		
Male	37	1
Do	37	1
Female	39	1
Do	36	1
Do	33	1
0.6 p.p.b. endrin:		
Female	35	1
Do	38	1
Do	41	2
Do	41	2
Male	36	2
0.4 p.p.b. endrin:		
Male	35	2
Female	39	2
Do	40	2
Do	38	A
Do	38	A
0.2 p.p.b. endrin:		
Male	30	A
Female	31	A
Male	40	A
Female	43	A
Do	43	A
Mean size	37.5	
Test S–13:		
0.5 p.p.b. endrin:		
Female	45	2
Male	46	2
Female	42	2
Male	40	2
Female	37	2
0.4 p.p.b. endrin:		
Female	35	2
Male	40	2
Do	39	2
Female	33	2
Male	39	3
0.3 p.p.b. endrin:		
Male	49	3
Female	36	3
Male	39	3
Female	40	4
Do	39	4
0.2 p.p.b. endrin:		
Female	42	A
Do	42	A
Male	47	A
Female	41	A
Male	39	A
Mean size	41.0	
Test S–14:		
0.5 p.p.b. endrin:		
Male	66	3
Female	59	3
Do	56	3
Male	55	3
Do	54	3
0.4 p.p.b. endcrin:		
Female	51	4
Male	75	A
Female	53	A
Male	56	A
Female	49	A
0.3 p.p.b. endcrin:		
Male	71	A
Female	63	A
Male	59	A
Female	57	A
Do	51	A
0.2 p.p.b. endrin:		
Female	53	A
Do	53	A
Male	61	A
Female	55	A
Do	52	A
Mean size	57.1	

APPENDIX TABLE 1.—*Results of acute-toxicity testing on bluntnose minnows*—Continued

	Standard length (mm.)	Number of days lived
Test S-15:		
0.5 p.p.b. endrin:		
Juvenile	32	2
Do	27	2
Do	30	2
Do	32	2
Do	33	2
0.4 p.p.b. endrin:		
Juvenile	27	4
Do	30	4
Do	33	4
Do	31	A
Do	30	A
0.3 p.p.b. endrin:		
Juvenile	30	3
Do	33	3
Do	26	4
Do	31	A
Do	32	A
0.2 p.p.b. endrin:		
Juvenile	24	A
Do	37	A
Do	32	A
Do	34	A
Do	35	A
Mean size	30.7	----------
Test S-16:		
0.5 p.p.b. endrin:		
Female	53	2
Do	41	2
Do	49	3
Do	36	3
Do	38	3
0.4 p.p.b. endrin:		
Female	50	3
Male	40	4
Female	42	4
Do	41	4
Do	52	A
Test S-16—Continued		
0.3 p.p.b. endrin:		
Male	41	4
Do	44	4
Female	43	4
Do	40	A
Do	52	A
0.2 p.p.b. endrin:		
Male	40	A
Female	36	A
Do	41	A
Male	39	A
Do	50	A
Mean size	43.2	----------
Test S-17:		
0.5 p.p.b. endrin:		
Male	57	3
Do	62	3
Do	64	3
Do	60	4
Do	65	A
0.4 p.p.b. endrin:		
Male	69	A
Do	62	A
Female	58	A
Unrecorded	----------	A
0.3 p.p.b. endrin:		
Female	53	A
Do	55	A
Do	57	A
Male	65	A
Female	54	A
0.2 p.p.b. endrin:		
Female	55	A
Male	56	A
Do	65	A
Female	55	A
Do	52	A
Mean size	58.9	----------

APPENDIX TABLE 2.—*Results of tests on oxygen consumption in the bluntnose minnow*

[Consumption is expressed in ml. per gram per hour. All testing was done at temperatures between 23° and 24° C.]

Test O–1: A 0.7-gram female, 38 mm. standard length. Consumption was measured at 7 a.m., 12 hours after the fish was fed.

Normal consumption	*Consumption in 0.4 p.p.b. endrin*
0.366	0.674
.320	.469
.320	.435
.366	.366
.366	.213
.352	.366
	.366
Mean .348	.366
	.160
	.320
	.275
	.310
	.354
	.443
	Test discontinued
	Mean .366

Test O–2: A 4.2-gram male, 70 mm. standard lengh. Consumption was measured daily at 7 a.m., 12 hours after feeding.

Normal consumption	*Consumption in 0.5 p.p.b. endrin*
0.210	0.184
.215	.230
.223	.267
.209	.329
.244	Fish died
.220	
.279	Mean .253
.215	
.215	
.188	
Mean .222	

APPENDIX TABLE 2.—*Results of tests on oxygen consumption in the bluntnose minnow*—Continued

Test O–4: A 5.0-gram male, 73 mm. standard length. Consumption was measured at 7 a.m. daily, 24 hours after feeding.

Normal consumption	*Consumption in 0.4 p.p.b. endrin*
0.135	0.285
.181	.219
.198	.305
.221	.316
.272	Fish died
.266	
.186	Mean__ .281
.218	
.280	
.175	
.272	
.299	
.169	
.249	
.327	
.266	
.270	
.150	
Mean__ .230	

Test O–5: A 2.0-gram female, 56 mm. standard length. Consumption was measured daily at 7 a.m. The fish was not fed.

Normal consumption	*Consumption in 0.4 p.p.b. endrin*
0.181	0.073
.133	.104
.182	.151
.074	Fish died
.044	
.236	Mean__ .109
.042	
.021	
.127	
.094	
.106	
.130	
.134	
.080	
Mean__ .113	

Test O–6: A 2.6-gram female, 60 mm. standard length. Consumption was measured twice daily at 7 a.m. and at 6 p.m. The fish was not fed.

Normal consumption	*Consumption in 0.3 p.p.b. endrin*
0.176	0.217
.185	.209
.193	.217
	.217
Mean__ .185	.194
	.180
	.173
	.184
	.184
	.147
	.137
	Mean__ 0.187

Test O–7: A 3.0-gram male, 61 mm. standard length. Consumption was measured twice daily, at 7 a.m. and 6 p.m. The fish was not fed.

Normal consumption	*Consumption in 0.5 p.p.b. endrin*
0.181	0.219
.191	.207
.228	.184
.197	.233
.224	Fish died
Mean__ .204	Mean__ .211

Test O–8: A 3.0-gram male, 61 mm. standard length. Consumption was measured twice daily, at 7 a.m. and 6 p.m. Fish was not fed and was starved seven days before the test was begun.

Normal consumption	*Consumption in 0.5 p.p.b. endrin*
0.142	0.116
.132	.136
.107	.144
.130	.164
	.241
Mean__ .128	Fish died
	Mean__ .160